

(19) 日本国特許庁 (J P)

(12) 公開特許公報 (A)

(11) 特許出願公開番号

特開平8-47392

(43) 公開日 平成8年(1996)2月20日

(51) Int.Cl. ⁶	識別記号	庁内整理番号	F I	技術表示箇所
C 1 2 N 15/09	Z N A			
C 0 7 K 7/08		8318-4H		
14/415		8318-4H		
// A 6 1 K 39/36	A B F			
		9281-4B	C 1 2 N 15/ 00	Z N A A
			審査請求 未請求 請求項の数12	F D (全 17 頁) 最終頁に続く

(21) 出願番号 特願平6-297840

(22) 出願日 平成6年(1994)11月7日

(31) 優先権主張番号 特願平5-276773

(32) 優先日 平5(1993)11月5日

(33) 優先権主張国 日本 (J P)

(31) 優先権主張番号 特願平6-134868

(32) 優先日 平6(1994)5月26日

(33) 優先権主張国 日本 (J P)

(71) 出願人 000006138

明治乳業株式会社

東京都中央区京橋2丁目3番6号

(72) 発明者 曾根 敏雄

神奈川県小田原市成田540番地 明治乳業

株式会社ヘルスサイエンス研究所内

(72) 発明者 小宮山 直樹

神奈川県小田原市成田540番地 明治乳業

株式会社ヘルスサイエンス研究所内

(72) 発明者 紀 光助

神奈川県小田原市成田540番地 明治乳業

株式会社ヘルスサイエンス研究所内

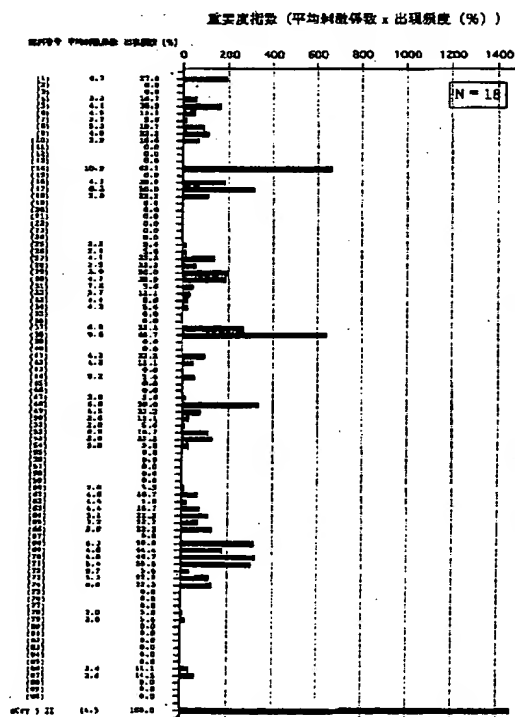
(74) 代理人 弁理士 吉田 研二 (外2名)

(54) 【発明の名称】 スギ花粉アレルゲンCry j I I エピトープ

(57) 【要約】

【目的】 スギ花粉症の診断、予防および治療に有用なスギ花粉アレルゲンCry j IIの少なくとも一つのエピトープ、特にT細胞エピトープを含むタンパク質またはペプチドを提供する。

【構成】 スギ花粉アレルゲンCry j IIをコードするcDNAをクローニングし、Cry j IIの全アミノ酸配列を明らかにした。更に、該アミノ酸配列全長にわたってオーバーラップペプチドを合成し、スギ花粉症患者由来のCry j II T細胞ラインを用いて、T細胞エピトープを含むオーバーラップペプチドを同定した。



1

【特許請求の範囲】

【請求項1】 配列表1記載のアミノ酸配列の全部または一部を含み、少なくとも1つのエピトープを保持するタンパク質またはペプチド。

【請求項2】 請求項1記載のタンパク質またはペプチドをコードするDNA。

【請求項3】 配列表3記載の塩基配列の全部または一部を含むことを特徴とする、請求項2記載のDNA。

【請求項4】 配列表2記載のアミノ酸配列の全部または一部を含み、少なくとも1つのエピトープを保持するタンパク質またはペプチド。 10

【請求項5】 請求項4記載のタンパク質またはペプチドをコードするDNA。

【請求項6】 配列表4記載の塩基配列の全部または一部を含むことを特徴とする、請求項5記載のDNA。

【請求項7】 T細胞エピトープであることを特徴とする、請求項1または4記載のタンパク質またはペプチド。

【請求項8】 下記のそれぞれのアミノ酸配列の全部または一部を含むことを特徴とする、請求項7記載のタンパク質またはペプチド。 20

1. Ala Ile Asn Ile Phe Asn Val Glu Lys Tyr Gly Ala Val Gly Asp

4. Gly Lys His Asp Cys Thr Glu Ala Phe Ser Thr Ala Trp Gln Ala

5. Thr Glu Ala Phe Ser Thr Ala Trp Gln Ala Ala Cys Lys Asn Pro

6. Thr Ala Trp Gln Ala Ala Cys Lys Asn Pro Ser Ala Met Leu Leu

7. Ala Cys Lys Asn Pro Ser Ala Met Leu Leu Val Pro Gly Ser Lys 30

8. Ser Ala Met Leu Leu Val Pro Gly Ser Lys Lys Phe Val Val Asn

9. Val Pro Gly Ser Lys Lys Phe Val Val Asn Asn Leu Phe Phe Asn

10. Lys Phe Val Val Asn Asn Leu Phe Phe Asn Gly Pro Cys Gln Pro

14. Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser Trp Lys

16. Pro Ala Ser Trp Lys Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys 40

17. Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr

18. Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu Met Gly Lys Gly

25. Glu Ile Cys Asn Asp Arg Asp Arg Pro Thr Ala Ile Lys Phe Asp

26. Arg Asp Arg Pro Thr Ala Ile Lys Phe Asp Phe Ser Thr Gly Leu

27. Ala Ile Lys Phe Asp Phe Ser Thr Gly Leu Ile Ile 50

2

e Gln Gly Leu

28. Phe Ser Thr Gly Leu Ile Ile Gln Gly Leu Lys Leu Met Asn Ser

29. Ile Ile Gln Gly Leu Lys Leu Met Asn Ser Pro Glu Phe His Leu

30. Lys Leu Met Asn Ser Pro Glu Phe His Leu Val Phe Gly Asn Cys

31. Pro Glu Phe His Leu Val Phe Gly Asn Cys Glu Gly Val Lys Ile

32. Val Phe Gly Asn Cys Glu Gly Val Lys Ile Ile Gly Ile Ser Ile 10

33. Glu Gly Val Lys Ile Ile Gly Ile Ser Ile Thr Ala Pro Arg Asp

34. Ile Gly Ile Ser Ile Thr Ala Pro Arg Asp Ser Pro Asn Thr Asp

37. Gly Ile Asp Ile Phe Ala Ser Lys Asn Phe His Leu Gln Lys Asn

38. Ala Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr Gly

41. Asp Asp Cys Val Ala Ile Gly Thr Gly Ser Ser Asn Ile Val Ile 20

42. Ile Gly Thr Gly Ser Ser Asn Ile Val Ile Glu Asp Leu Ile Cys

44. Glu Asp Leu Ile Cys Gly Pro Gly His Gly Ile Ser Ile Gly Ser

47. Leu Gly Arg Glu Asn Ser Arg Ala Glu Val Ser Tyr Val His Val

48. Ser Arg Ala Glu Val Ser Tyr Val His Val Asn Gly Ala Lys Phe

49. Ser Tyr Val His Val Asn Gly Ala Lys Phe Ile Asp Thr Gln Asn 30

50. Asn Gly Ala Lys Phe Ile Asp Thr Gln Asn Gly Leu Arg Ile Lys

51. Ile Asp Thr Gln Asn Gly Leu Arg Ile Lys Thr Trp Gln Gly Gly

52. Gly Leu Arg Ile Lys Thr Trp Gln Gly Gly Ser Glu Met Ala Ser

53. Thr Trp Gln Gly Gly Ser Gly Met Ala Ser His Ile Ile Tyr Glu

54. Ser Gly Met Ala Ser His Ile Ile Tyr Glu Asn Val Glu Met Ile 40

60. Ala Ser Ala Cys Gln Asn Gln Arg Ser Ala Val Gln Ile Gln Asp

61. Asn Gln Arg Ser Ala Val Gln Ile Gln Asp Val Thr Tyr Lys Asn

62. Val Gln Ile Gln Asp Val Thr Tyr Lys Asn Ile Arg Gly Thr Ser

63. Val Thr Tyr Lys Asn Ile Arg Gly Thr Ser Ala Thr Ala Ala Ala

64. Ile Arg Gly Thr Ser Ala Thr Ala Ala Ala Ile Glu 50

n Leu Lys Cys

65. Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys Ser Asp Ser Met Pro

66. Ile Gln Leu Lys Cys Ser Asp Ser Met Pro Cys Lys Asp Ile Lys

68. Cys Lys Asp Ile Lys Leu Ser Asp Ile Ser Leu Lys Leu Thr Ser

69. Leu Ser Asp Ile Ser Leu Lys Leu Thr Ser Gly Lys Ile Ala Ser

70. Leu Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn

71. Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn Ala Asn Gly Tyr Phe

72. Cys Leu Asn Asp Asn Ala Asn Gly Tyr Phe Ser Gly His Val Ile

73. Ala Asn Gly Tyr Phe Ser Gly His Val Ile Pro Ala Cys Lys Asn

74. Ser Gly His Val Ile Pro Ala Cys Lys Asn Leu Ser Pro Ser Ala

78. Lys Ser His Lys His Pro Lys Thr Val Met Val Glu Asn Met Arg

79. Pro Lys Thr Val Met Val Glu Asn Met Arg Ala Tyr Asp Lys Gly

86. Cys Ser Pro Cys Lys Ala Lys Leu Val Ile Val His Arg Ile Met

87. Ala Lys Leu Val Ile Val His Arg Ile Met Pro Glu Glu Tyr Tyr

【請求項9】 下記のそれぞれのアミノ酸配列の全部または一部を含むことを特徴とする、請求項7記載のタンパク質またはペプチド。

14. Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser Trp Lys

17. Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr

29. Ile Ile Gln Gly Leu Lys Leu Met Asn Ser Pro Glu Phe His Leu

38. Ala Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr Gly

48. Ser Arg Ala Glu Val Ser Tyr Val His Val Asn Gly Ala Lys Phe

68. Cys Lys Asp Ile Lys Leu Ser Asp Ile Ser Leu Lys Leu Thr Ser

70. Leu Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn

71. Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn Ala Asn Gly Tyr Phe

【請求項10】 請求項8または9に記載のタンパク質またはペプチドをコードするDNA。

【請求項11】 請求項8または9に記載のタンパク質またはペプチドと免疫学的に交差反応性を有するタンバ

ク質またはペプチド。

【請求項12】 請求項11に記載のタンパク質またはペプチドをコードするDNA。

【発明の詳細な説明】

【0001】

【産業上の利用分野】 本発明は、スギ花粉症の診断、予防もしくは治療に有用な、スギ花粉アレルゲンCry j IIのエピトープ、特にT細胞エピトープを含むタンパク質またはペプチド、または該タンパク質またはペプチドをコードするDNAに関する。

【0002】

【従来の技術】 スギ花粉症は、スギ花粉が飛散する春先にはほぼ全国的に観察されるアレルギー性疾患であり、くしゃみや鼻汁、目のかゆみ等を伴うアレルギー症状を呈する。その患者数は、1970年以降急激に増加しており、現在全国民の10%弱に当たる約一千万人がスギ花粉症に苦しめられている。

【0003】 アレルギー性疾患を形成するアレルギー反応は、R. G. H. Gell とR. R. A. Coombs によりI型～IV型の4種に分類されており、スギ花粉症はI型に属する。I型アレルギーの発症機序は以下の通りである。

【0004】 アレルギー反応を引き起こす分子をアレルゲン（本明細書では抗原ともいう）というが、花粉の場合このアレルゲンがタンパク質抗原である。これらの外来タンパク質抗原が体内に侵入すると、抗原提示細胞（マクロファージ）に取込まれ、タンパク分解酵素によって分解されてペプチド断片になり、主要組織適合抗原複合体（Major Histocompatibility Complex: MHC）クラスII分子（ヒトではHLAクラスII分子）と結合した状態で、細胞膜上に提示される。HLAクラスII分子は多型性を示すが、CD4⁺T細胞のレセプターは、HLAクラスII分子と結合した抗原ペプチドを、そのHLAクラスII分子の多型性を示す部分と共に認識し、抗原特異的に活性化される。活性化されたCD4⁺T細胞は、Th0細胞、Th1/Th2細胞に分化し、種々のサイトカインを産生する。その際、それぞれの細胞のサイトカイン産生パターンは異なっており、Th1はIL-2、IFN γ を、Th2はIL-4、IL-5、IL-10等を、Th0は両者のサイトカインを産生する。

【0005】 一方、B細胞は細胞表面にIgMあるいはIgDを表現しており、抗原を細胞内に取込むことによって活性化される。その際、Th2から産生されるサイトカインの作用によって、活性化されたB細胞は抗体産生細胞にまで分化増殖し、抗原特異的な免疫グロブリンE（IgE）を産生する。このようにして産生されたIgEは、気道あるいは鼻粘膜組織中のマスト（肥満）細胞や血液中の好塩基球にIgEレセプターを介して強固に結合し、感作が成立した状態になる。

【0006】 再び、アレルゲンが体内に侵入すると、1分子のアレルゲンは、直ちにマスト細胞や好塩基球上の

2 分子以上のIgE と結合し、架橋構造を形成する。その結果、IgE 分子と結合しているレセプター同士が会合し、これが引き金となって、細胞膜内の幾種類もの酵素が活性化され、ヒスタミンやプロスタグランジン、ロイコトリエンといった種々の化学伝達物質が細胞から放出される。これらの化学伝達物質が鼻粘膜や気道などの局所に作用して、色々なアレルギー症状を引き起こす。

【0007】なお、T 細胞によって認識されるエピトープをT 細胞エピトープ、B 細胞によって認識されるエピトープをB 細胞エピトープという。

【0008】アレルゲンのエピトープは、I 型アレルギーの発症及び増悪に直接関与していると考えられるので、アレルゲンのエピトープを同定することは、I 型アレルギーの診断、予防及び治療に有用である。

【0009】スギ花粉の主要アレルゲンは、安枝らによって単離精製され、Sugi Basic Protein (SBP) と命名された (Yasueda, H., et al., J. Allergy Clin. Immunol. 71, 77-86, 1983)。このSBP は、分子量が45~50kDa で、WHO の命名法に従い現在Cry j I と呼ばれている。更にその後、Cry j I の分離精製の過程で、Cry j I とは抗原性の異なる、分子量が37kDa のCry j IIが分離された (Tanai, M. et al. FEBS Letters 239, 329-332, 1988, Sakaguchi, M. et al. Allergy 45, 309-312, 1990)。

【0010】これらの結果、Cry j I とCry j IIとは全く異なるタンパクであることが明らかとなったが、スギ花粉症患者では、Cry j I とCry j IIの両者が反応していることが報告された。すなわち、145 名のスギ花粉症患者血清中、134 名 (92.4%) の血清がCry j I 及びCry j IIと反応し、6 名 (4.1%) の血清がCry j I とのみに反応し、5 名 (3.4%) の血清がCry j IIとのみ反応することが判明した (1993年第43回日本アレルギー学会、橋本ら、日獣大、予研、国立相模原病院、林原生化研)。つまり、スギ花粉症の発症には、Cry j I 及びCry j IIのどちらも重要であることが示された。

【0011】Cry j I については、それをコードするcDNAがクローニングされ、その推定アミノ酸配列に基づき、T 細胞エピトープを含むペプチドが同定されている (W094/01560, "ALLERGENIC PROTEINS AND PEPTIDES FROM JAPANESE CEDAR POLLEN")。Cry j IIについては、N 末端のアミノ酸配列のAla、Ile、Asn、Ile、Phe、Asn、Val、Glu、Lys 及びTyr の10アミノ酸残基が報告されている (Sakaguchi, M., et al., Allergy 45, 309-312, 1990) に過ぎない。

【0012】

【発明が解決しようとする課題】本発明は、スギ花粉症の診断、予防及び治療に有用な、スギ花粉アレルゲンCry j IIの少なくとも1つのエピトープ、特にT 細胞エピトープを含むタンパク質またはペプチド、または該タンパク質またはペプチドをコードするDNAを提供するこ

とを目的とする。

【0013】

【課題を解決するための手段】本願発明者らは、上記課題を解決するために、

(1)Cry j II の全アミノ酸配列 (一次構造) の解明

(2)Cry j II の全アミノ酸配列をカバーするオーバーラップペプチドの作製

(3)Cry j II アレルゲンを特異的に認識するT 細胞ラインを個人別に樹立

10 (4) 抗原提示細胞 (B 細胞株) の樹立

(5)T細胞エピトープを含むオーバーラップペプチドの同定

を行い、本発明を完成した。なお、本発明でいうエピトープは、T 細胞エピトープに限られるものではないが、以下T 細胞エピトープについて詳述する。これらの各ステップを以下に説明する。

【0014】(1)Cry j II の全アミノ酸配列の解明

① cDNAのクローニング

a. RNAの抽出

20 RNA を抽出する際、通常、初期段階で蛋白質を除去する。このため一般的な方法として、フェノール抽出方法、グアニジウム塩、界面活性剤、尿素などの蛋白質変性剤などを用いる方法がある。

【0015】スギ花粉からのRNA 抽出は、Breitenederら (Int. Arch. Allergy Appl. Immunol. 87: 19-24, 1988) の方法に改良を加えて行うことができる。

【0016】スギ花粉を、10~20倍量の抽出緩衝液 (10mM LiCl、10mMNa₂EDTA、1%SDS、20%メルカプトエタノール、100mM Tris-HCl pH 9.0) に懸濁し、これに等量のフェノールとクロロホルムの混液 (フェノール:クロロホルム:イソアミルアルコール=24:24:1) を加えホモジェナイズする。次いで遠心 (10,000g、10~15分) し、フェノール・クロロホルム層と、水層の二層に分離する。このとき変性した蛋白質はフェノール・クロロホルム層に、核酸は水層に移行する。水層にフェノール・クロロホルム混液を加え、振盪し水層に残存している蛋白質などの不純物をフェノール・クロロホルム層に移行させ除去する。このような操作を2回繰り返す。

40 【0017】得られた水層からRNA を抽出するには、高濃度のLiCl (2~4M) またはCH₃COONa (3M)が存在するとDNA 及び蛋白質は上清に残り、tRNA以外のRNA は沈殿する性質を利用する。水層に同量の2~4MのLiClを添加し、RNA を沈殿させる。次いでこの水層を水に溶解し、0.1~0.3 容の冷エタノール (-20℃) を加え、RNA を沈殿させる (エタノール沈殿)。次いで遠心 (10,000g、30分) して沈殿を回収し、水に溶解して全RNA 分画を得る。

【0018】b. mRNA の調製とcDNAの合成

50 Cry j IIのmRNAは、3'末端にポリ(A) 鎖を持つので、こ

7

8

れと相補するリガンドとして12~18塩基のデオキシチミン(dT)を結合したオリゴdTセルロースカラム(Clontech Laboratories Inc. 社製、CA, USA)にmRNAを吸着される。スギ花粉RNAに緩衝液(3M NaCl、1mM EDTA、10mM Tris-HCl、pH7.4)を加えてmRNAをカラムに吸着させる。mRNAは、ベッド体積の2~3倍量のNaClを含まない緩衝液(1mM EDTA、10mM Tris-HCl、pH7.4)で溶出する。

【0019】得られたmRNAからのcDNAライブラリーの作製は、現在市販されているファージをベクターに用いたcDNAライブラリー作製キット(Amersham International plc. 社製、Buckinghamshire、England)を用いて行うことができる。

【0020】c. Cry j II cDNAのスクリーニング
Cry j IIのN末端アミノ酸10残基が既に判明しているが、このアミノ酸配列から推定した塩基配列を持つ合成DNAをプローブとして、Cry j II cDNAをスクリーニングすることができる。プローブに用いるDNAを合成する場合、可能性のあるコドンを含むオリゴヌクレオチドを全て合成するよりも、可能性のある複数のコドン配列に対してハイブリダイズするようなオリゴヌクレオチドを設計することが望ましい。この合成オリゴヌクレオチドプローブの5'末端を[γ -³²P]ATPとポリヌクレオチドキナーゼによって標識し、ブランクハイブリダイゼーション法により、前記cDNAライブラリーから陽性クローンをスクリーニングする。

【0021】得られた陽性クローンよりファージDNAを調製し、挿入cDNA断片を分離し、pUC18等のプラスミドにサブクローンする。必要に応じてオリゴヌクレオチドプライマーを合成し、Sanger法等により塩基配列を決定し、クローンを同定する。本発明者らが単離したCry j II cDNAの全長の塩基配列を、配列番号5に示す。

【0022】Cry j IIをコードするcDNAは、全体で1733bpからなり、翻訳開始と想定されるコドン(45~47位のヌクレオチドATG)から終止コドン(1587~1589位のヌクレオチドTAA)に至るオープンリーディングフレームを含み、514アミノ酸をコードしている。オープンリーディングフレーム部分の塩基配列を配列番号3に示し、また該塩基配列がコードするアミノ酸配列を配列番号1に示す。配列番号3で示される塩基配列には、個体間での対立遺伝子変異による多型性(polymorphism)及びその結果としてのアミノ酸配列の変異が考えられるがそのような変異を有するCry j IIの塩基配列及びアミノ酸配列も本発明に包含される。207~236位のDNA配列のコードするアミノ酸配列はAla、Ile、Asn、Ile、Phe、Asn、Val、Glu、Lys、Tyrであり、成熟型Cry j IIのN末端アミノ酸配列(Sakaguchi, M., et al., Allergy 45, 309-312, 1990)と一致する。N末端の54アミノ酸は、他のシグナルペプチドに見られる疎水性アミノ酸に富み、また成熟型Cry j IIに含まれていないことからシグナルペプチドと考えられる。

10

20

30

40

50

【0023】207位から終止コドン1587~1589位までのDNA配列がコードするCry j IIは、N末端のAlaからC末端のProまで460個のアミノ酸残基からなり、成熟型Cry j IIと考えられる。該成熟型Cry j IIに対応する塩基配列を配列番号4に、該塩基配列にコードされるアミノ酸配列を配列番号2に示す。配列番号2に示すアミノ酸配列からなるCry j IIの理論上の分子量は50,444Daである。一方、天然の成熟型Cry j IIは、還元条件下のSDS-ポリアクリルアミド電気泳動(SDS-polyacrylamide gel electrophoresis)で45KDaの位置にそのバンドが現れる(Sakaguchi, M., et al., Allergy 45, 309-312, 1990)。このことから、Cry j IIのC末端はプロセッシングを受けているものと考えられる。また成熟Cry j IIのアミノ酸配列の中には、N-グリコシド結合の可能性のあるAsn-X-Ser/Thrが存在する。

【0024】Cry j IIをコードするDNAの全長またはその一部を含むDNAは、蛍光標識、放射性標識あるいは酵素標識等によって標識することにより、生化学検査または関連蛋白質若しくは類似の配列を含む蛋白質をコードするDNAのスクリーニング等のためのプローブ、プライマーとして使用できる。また発現ベクターに接続して、少なくとも1つのエピトープを含むタンパク質またはペプチドを発現させることもできる。

【0025】②組換えCry j II(rCry j II)の発現
rCry j IIまたは少なくとも一つのCry j IIのエピトープを含む組換えタンパク質またはペプチドは、それぞれをコードするcDNAを発現ベクターに組み込み、大腸菌、昆虫細胞、酵母または哺乳動物に導入し、培養することにより得ることができる。しかし、大腸菌などの原核細胞を使う発現系は、適切な糖鎖の付加(glycosylation)が行われないうえに、rCry j IIの発現には酵母などの真核細胞を使用することが好ましい場合がある。

【0026】Cry j IIの幾つかの発現システムの例を以下に示す。

【0027】a. 大腸菌での発現

T7ファージのプロモーターとRNAポリメラーゼを用いる系(F. W. Studier, A. H. Rosenberg, J. J. Dunn, J. W. Dubendonff, "Methods in Enzymology", ed. by D. D. V. Goeddel, vol. 185, p. 60, Academic Press, New York, 1990)は、極めて発現の成功率が高いので、本発明に好適に使用できる。この系は、T7ファージのポリメラーゼ遺伝子を持つ大腸菌宿主BL21(DE3)に、T7ファージプロモーターの下流のマルチクローニングサイトに目的の遺伝子を挿入した組換えプラスミドを導入して、IPTG存在下で、目的の遺伝子を発現させるシステムである。例えば発現ベクターとしてpGEMEX-1(Promega社)などが使用できる。

【0028】また、目的の蛋白質を、大量発現可能な蛋白質と融合させて発現させる系が市販されており、これらの系は精製にアフィニティーカラムが使え、精製効率

がよく、本発明に好適に使用できる。例えば、融合蛋白質にβ-ガラクトシダーゼを有する発現ベクターpUEX(Amersham)を用いると、rCry j IIはβ-ガラクトシダーゼとの融合蛋白質として得られ、アフィニティカラムで効率よく精製することが出来る。また、グルタチオンS-トランスフェラーゼを有するpGEX(Pharmacia)や、マルトース結合蛋白質を用いたpMAL(New England Biolabs, Beverly, MA)などは、その融合部位に血液凝固因子Xaの切断部位が導入されており、Cry j IIを分離することができる。

【0029】b. 酵母での発現

酵母を宿主とする系は発現産物のグリコシレーションが可能であり、このことは糖蛋白質であるCry j IIの発現に好都合である。例えば酵母による異種蛋白質の発現系としては、ピキア酵母を宿主として用いる方法が知られており(特開昭61-108383号公報、特開昭61-173781号公報、特開昭63-44899号公報、特開平1-128790号公報等)、本発明に好適に使用できる。その他の酵母による発現系については、D. Emr Scott, "Methods in Enzymology", ed. by D. V. Goeddel, vol. 185, p.231, Academic Press, New York (1990)に詳述されており、本発明で使用できる。

【0030】c. 昆虫細胞での発現

昆虫細胞中を宿主とする系は発現産物のグリコシレーションが可能である。バキュロウイルスを用いた外来遺伝子発現システムは市販されており(PharMingen, San Diego, CA, USA)、本発明に好適に使用できる。このシステムについては、Luckow, V. A. らのTrends in the Development of Baculovirus Expression Vector, Bio/Technology (1987年9月11日)に記載されている。

【0031】d. 哺乳動物細胞での発現

哺乳類プロモーター(例えばメタロチオネイン)、ウイルスプロモーター(例えばSV40初期プロモーター)等を持つ発現ベクターに組み込み、哺乳動物細胞に導入することにより高発現させることができる。

【0032】(2) オーバーラップペプチドの合成

花粉症患者のT細胞が認識するCry j IIのT細胞エピトープを分子レベルで解明するために、配列番号2に記載のCry j II cDNAのコードするアミノ酸配列に基づき、N末端のAlaからC末端のProに至る全460アミノ酸残基をカバーするオーバーラップペプチドを作製する。これらのオーバーラップペプチドは、市販されているペプチド自動合成装置により容易に合成することができる。これらのオーバーラップペプチドの中から、少なくとも一つのエピトープを含むペプチドを同定する。特に、T細胞エピトープを同定するためには、花粉症患者の末梢血リンパ球から、Cry j IIを特異的に認識し増殖応答するT細胞ラインを樹立する必要がある。一般に、患者毎に反応するT細胞エピトープが異なるので、患者毎にT細胞ラインを樹立することが望ましい。

【0033】(3) T細胞ラインの樹立

Cry j II抗原特異的なT細胞ラインを樹立するには、通常患者の末梢血リンパ球をCry j II抗原の存在下、7日間程度培養して抗原刺激によりT細胞を活性化し、さらに、活性化T細胞を、抗原と抗原提示細胞と共に7日間培養することを数回繰り返して抗原刺激することにより、抗原特異的なT細胞ラインを作製することができる。しかしながら、T細胞が増殖因子のIL-2の存在下でよく増殖している場合は、抗原刺激は最初だけにすることが望ましい。T細胞ラインを数度抗原刺激すると、増殖率の高いT細胞が選択的に取れ、T細胞エピトープを含むペプチドを同定する場合において、エピトープによっては十分な増殖応答を示さない場合が生じる。

【0034】使用する抗原としては、原理的には天然型Cry j II抗原が望ましいが、極微量しかスギ花粉から抽出できないことから、組換えCry j II(rCry j II)あるいはオーバーラップペプチドの混合物も好適に使用できる。rCry j IIは、大腸菌で発現させ精製したものが利用できる。

20 【0035】(4) 抗原提示細胞(B細胞株)の樹立

抗原提示細胞としては、T細胞ラインと同一人の末梢血リンパ球を、マイトマイシンC処理あるいは放射線照射して増殖能力を失わせたものが望ましい。しかし、採血回数が多くなるため、Epstein-Barr virus (EBV)を自己のBリンパ球に感染させトランスフォーメーションを起こさせたものは、in vitroで増殖し続けリンパ芽球様細胞株(B細胞株)となるので、このB細胞株を抗原提示細胞として用いてもよい。B細胞株の樹立方法は既に確立されている[組織培養の技術第二版、187-191頁、日本組織学会編(1988.8.10)]。

30 【0036】(5) T細胞エピトープを含むオーバーラップペプチドの同定

それぞれの患者固有のT細胞ラインが認識する、T細胞エピトープを含むペプチドは以下のようにして同定される。ここで「認識する」という意味は、T細胞レセプターが抗原エピトープ(MHC分子を含めて)と特異的に結合し、その結果、T細胞が活性化されることを意味し、活性化の状態は、リンホカインの産生や、DNAの合成を[³H]チミジンの取込み量を指標として測定することにより観察される。すなわち、T細胞ラインとマイトマイシンC処理した同一人のB細胞株とを、96穴平底プレートに播種し、オーバーラップペプチドと共に混合培養し、[³H]チミジンの取込み量(cpm)を液体シンチレーションカウンターで測定する。その際、[³H]チミジンの取込みは、個々の培養系で異なるため、例えば、個々のペプチドに対するT細胞ラインの[³H]チミジン取込み量(cpm)を、抗原を添加していないコントロールの[³H]チミジン取込み量(cpm)で除した数(stimulation index: SI)が2以上をT細胞エピトープを含むペプチドと同定する。同定されたT細胞エピ

トープを含むペプチドは、図4に列挙されている。

【0037】このようにして得られた本発明のCry j IIの少なくとも一つのT細胞エпитープを含むペプチドについては以下のことが考えられる。HLAクラスII分子と結合して抗原提示されるペプチドの長さは、ペプチドの解析結果(Chicz, R. M. et al.: J. Exp. Med., 178: 27-47, 1993)から、およそ10~34のアミノ酸残基からなるものと考えられるので、本発明のT細胞エпитープを含むペプチドはこのような長さのペプチドも含まれる。また、本発明のペプチドにアミノ酸置換、欠失あるいは付加などの修飾を行い、これらの修飾ペプチドに対する患者毎のT細胞ラインの増殖応答を測定することによって、本発明のCry j IIの少なくとも一つのT細胞エпитープを含むペプチドと免疫学的に同機能を有する修飾ペプチドを容易に作製することは、当業者が容易に実施しうることであり、これらの修飾ペプチドも本発明に包含される。

【0038】現在、減感作療法で使用されている減感作剤はスギ花粉から抽出された粗抗原であり、多量の多糖類を含んでいる。ロット差がかなりあり、一旦減感作療法を開始した後、ロットを変えるとアナフィラキシーを起こすことが稀にある。また、減感作の治療効果も、減感作治療が開始されて以来余り改善されておらず、減感作療法で著効と診断されるのは約30%の患者である。

【0039】本発明のT細胞のエピトープを含むペプチドのうち、花粉症患者の半分以上のT細胞ラインと反応する各々のペプチドは、これらの各ペプチドを単独もしくはいくつかを混合したペプチドを用いて減感作療法を行った場合には、治療した患者の半分以上で減感作が行える可能性がある。また、使用するペプチドは、化学的に合成されたペプチドであるため、アナフィラキシーのような副作用を生じる可能性は低くなると考えられる。例えば、図5は、18名の花粉症患者から樹立されたT細胞ラインがそれぞれ認識するオーバーラップペプチドを、重要度指数[「平均刺激係数」(「オーバーラップペプチド刺激によるT細胞ラインの[³H]チミジン取込み量(cpm)」を「抗原を添加しない場合の[³H]チミジン取込み量(cpm)」で割った値の平均値)と「出現頻度(%)」(「試験した全T細胞ライン」に対する「被験ペプチドをT細胞エピトープとして認識したT細胞ライン」の割合(%))とを乗じた値]で示したものであるが、図中の番号14、17、29、38、48、68、70および71のペプチドは平均刺激係数が約3.9以上である上、重要度指数が200を超えており、減感作治療に特に有効であると考えられる。

【0040】なお、本発明者が明らかにし、図5に示された少なくとも一つのT細胞エピトープを含むペプチドの中には、後述のB細胞エピトープを含むことが判明した2種類のペプチドと共通部分を有するペプチドは含まれていない。従って本発明のペプチドは、B細胞エピ

トープを刺激しないと考えられるので、減感作剤として実用化可能であると考えられる。

【0041】また、本発明のT細胞のエピトープを含むペプチドを経口投与して、経口免疫寛容を行うことも可能と考えられる。経口免疫寛容(経口減感作)は現在開発中の治療法であるが、効果を示す結果が報告され始めている。例えば、Myelin Basic ProteinのT細胞エピトープ(ペプチド配列21-40、71-90)をマウスに経口投与すると「Experimental Autoimmune Encephalomyelitis(略してEAE)発症」を抑制したことが報告されている[上野川修一、久恒辰博、八村敏志、経口免疫寛容の分子生物学、蛋白質核酸酵素、39、2090-2101(記載頁2098右、9-24行)1994年]。これらの例から、スギ花粉症においても、同定したT細胞エピトープペプチドをそのまま経口投与するか、あるいは胃で消化されないように何らかのカプセルに封入する等の工夫を行って経口投与すれば、免疫寛容状態になる可能性がある。スギ花粉飛散時期の前、具体的には12~1月期に経口的にエピトープペプチドを投与し、免疫寛容状態を誘導しておく。この状態だとスギ花粉が飛散して鼻粘膜に花粉が付着しても、症状が出ないか、あるいは症状が軽くなることが期待される。

【0042】さらにまた、本発明のT細胞のエピトープを含むペプチドに、アミノ酸置換、欠失あるいは付加などの修飾を加えたアナログペプチドを合成し、HLAクラスII分子には結合するが、T細胞には情報が伝わらないアナログペプチドを同定する。これらのペプチドは、例えば点鼻薬として患者に使用すれば、天然のT細胞エピトープを競合的に阻害するので、発症予防が期待される。

【0043】なお、本発明でいうエピトープには、B細胞エピトープも含まれる。B細胞エピトープの同定は、オーバーラップペプチドと患者血清IgE抗体との反応性の測定、オーバーラップペプチドによる患者血清と抗原との結合の阻害の検出等の公知の方法によって行うことができる(特開平6-69336号参照)。既に、1価のB細胞エピトープは、アレルギー反応の抑制に有用であることが知られている。これは、1価のB細胞エピトープは、肥満細胞または好塩基球上の対応するIgE分子と結合し、多価エピトープによるIgE分子架橋の形成を阻害することによるものと考えられている。本発明者らは、本発明のCry j IIの全アミノ酸配列をカバーするオーバーラップペプチドを合成し、これらのペプチドとスギ花粉症患者血清IgE抗体との反応を酵素抗体法で測定した結果、ペプチド「Gln Cys Lys Trp Val Asn Gly Arg Glu Ile Cys(アミノ酸配列113~123)」および「Cys Thr Ser Ala Ser Ala Cys Gln Asn(アミノ酸配列293~301)」はB細胞エピトープを含んでいることを明らかにした。このようなCry j IIのB細胞エピトープを含むペプチドは、スギ花粉症の診断、予防及び治療

に有用である。

【0044】

【実施例】以下本発明を実施例に基づいて詳細に説明するが、本発明はこれに限定されない。

【0045】＜スギ花粉の採取＞スギ花粉は静岡県及び神奈川県内で2月に伐採されたスギの枝に着花した雄花から採取した。Cry j II 抗原性精製用のスギ花粉は-70℃で保存し、RNA調製用のスギ花粉は液体窒素中で急速凍結した後、-70℃で保存した。

【0046】＜RNAの抽出＞Breitenederら(Int. Arch. Allergy Appl. Immunol. 87:19-24 1988)の方法を基にして改良を加えることによりスギ花粉からRNAを抽出した。

【0047】凍結保存したスギ花粉1gを氷冷した15mlの抽出緩衝液(100mM LiCl、10mM Na₂EDTA、1%SDS、20% 2-メルカプトエタノール、100mM Tris-HCl、pH 9.0)に懸濁し、さらに、15mlのフェノール：クロロフォルム：イソアミルアルコール(24:24:1)を添加した。この懸濁液をテフロンホモジェナイザーに移し、テフロンペステルをモーターで最高回転で回しながら、20～30ストロークホモジェナイズした。この後、遠心操作(10,000g、15分)で水層と有機層に分離して水層を得た。水層に同量のフェノール：クロロフォルム：イソアミルアルコールを加え、5分間振蕩の後、遠心分離(10,000g、15分)で水層を得た。同様の操作を2回繰り返し、さらに15mlのクロロフォルム：イソアミルアルコール(24:1)を用いて1回行った。得られた水層に同量の4M LiClを添加して-20℃で一晩放置した。凍結した溶液を室温で溶解し、遠心操作(20,000g、30分)で沈澱を得た。この沈澱を少量の滅菌蒸留水に溶解し、0.3容の3M CH₃COONa、pH 5.2と2.5容のエタノールを加え、-20℃で60分間放置した。遠心操作(10,000g、30分)により回収した沈澱を滅菌蒸留水に再溶解して全RNA分画とした。

【0048】＜スギ花粉mRNAの調製とcDNAの合成＞スギ花粉全RNA1mgを出発材料として同量の結合緩衝液(3M NaCl、1mM EDTA、10mM Tris-HCl、pH 7.4)を添加した後、オリゴdTセルロースを事前にバックしたスパンカラム(CLONETECH Laboratories Inc.社製、CA、USA)に吸着させ、溶出緩衝液(1mM EDTA、10mM Tris-HCl、pH 7.4)で溶出することにより約10μgのmRNAを精製した(CLONETECH Lab. Inc.社添付プロトコールに従った)。続いて、精製mRNA 5μgからcDNA合成システムプラス(Amersham International plc.社製、Buckinghamshare、England)を使用し、添付されているプロトコールに従ってcDNA約4μgを合成した。

【0049】＜オリゴヌクレオチドプローブの合成＞Cry j IIのN末端から10残基のアミノ酸配列を図1Aに示す。このアミノ酸配列から予想されるcDNAの配列は図1Bである。オリゴヌクレオチドプローブ(Oligo CJII)としてその配列に相補的に、また4カ所で2種類の塩基

を用いているので、合計16種類の混合物として合成した(図1C)。混合物として種類を減らすためにG:T塩基対を許容している。

【0050】＜Cry j II cDNAのクローニング＞cDNAライブラリーの作製はcDNAクローニングシステムλgt10(Amersham International plc.社製、Buckinghamshare、England)を使用し、添付されているプロトコールに従って行った。上述のcDNA 1μgをλgt10に組み込みcDNAライブラリーを作製した。約50万のライブラリーのうち約5,000のクローンを直径150mmのプレート1枚にまいた。スクリーニングのためのプローブは上記のオリゴヌクレオチド(Oligo CJII)をT4 polynucleotide kinaseにより[γ-³²P]ATP (7,000Ci/mmol ICN Biochemicals, Inc.社製)で標識して用いた。ファージDNAを固定化したニトロセルロースフィルターを5×SSPE(1×SSPE:0.18M NaCl、10mMリン酸ナトリウム、1mM EDTA)、5×FBP(1×FBP:0.02% Ficoll、0.02%牛血清アルブミン、0.02% ポリビニルピロリドン)、0.3%SDS、100 μg/ml tRNAを含む溶液に48℃1時間以上浸すことによりプレハイブリダイズした。この後ニトロセルロースフィルターを新たに調製した同溶液に浸し、³²Pラベルしたプローブ(Oligo CJII)を加えて48℃で一晩ハイブリダイゼーションを行った。この後フィルターを6×SSC(1×SSC:0.15M NaCl、0.015Mクエン酸ナトリウム)と0.1%SDSを含む溶液で室温30℃、48℃5分洗浄した後、オートラジオグラフィーを行った。4個の強いシグナルが検出され、そのうちの1つのファージDNAを抽出し、制限酵素EcoRIで切断したところ約1.7KbpのDNA断片が挿入されていることが判明した。挿入断片をpUC18にサブクローニングし、キロシークエンスデレーションキット(宝酒造社製)を用いてデレーションミュータントを作製し全塩基配列の決定に用いた。塩基配列は合成プライマーと色素標識ジデオキシターミネーターを用いてプライマー伸長反応を行い、自動シークエンサー(モデル370A、Applied Biosystems、Japan)で判読することにより決定した。決定されたcDNA全塩基配列を配列番号5に示す。また、オープンリーディングフレームのみの塩基配列を配列番号3に(該塩基配列がコードするアミノ酸配列を配列番号1に)、成熟Cry j IIをコードする塩基配列を配列番号4に(該塩基配列がコードするアミノ酸配列を配列番号2に)示す。

【0051】＜組換えCry j IIの大腸菌での発現＞Promega社より市販されている大腸菌発現ベクターpGEMEX-1はT7プロモーター、T7 gene10のコーディングシークエンスおよびT7ターミネーターをもち、オープンリーディングフレームをT7 gene10の下流のマルチクローニングサイトに挿入してT7 RNAポリメラーゼを発現する大腸菌(例BL21(DE3))に導入することにより高発現を行うベクターである。Cry j II cDNAをBamHI(cDNAの両端に連結したアダプターはBamHIサイトを含む)で消化して

cDNAフラグメントを切り出しpGEMEX-1のBamHI サイトに組み込みCry j IIの発現ベクターpEXCIIを構築した。pEXCIIはT7 gene10 発現産物 (23kD) とCry j II蛋白質 (50kD) との融合蛋白質 (T7 Cry j II、73kD) を発現し得る。pEXCIIを大腸菌BL21(DE3) に導入した形質転換体を培養しIPTGでT7 RNAポリメラーゼを誘導してCry j IIの発現を行った。発現した大腸菌の細胞抽出液をSDS ポリアクリルアミドゲル電気泳動にかけた。pEXCIIを保持するBL21(DE3) には、約73kDのT7 Cry j IIと思われるバンドが見られた。しかし、対照のpGEMEX-1を保持するBL21(DE3) または親株BL21(DE3) には、これらのバンドは見られなかった。

【0052】<Cry j IIとT7 gene10 との融合タンパク質 (T7Cry j II) のスギ花粉症患者血清との反応性>T7 Cry j II を発現した大腸菌の抽出液を、SDS ポリアクリルアミドゲル電気泳動した後、Millipore 社製PVDF膜にウェスタンブロッティング (Western Blotting) し、スギ花粉症患者5人、健康人3人の血清との反応性を検討した。対照としてpGEMEX-1を保持するBL21およびT7 gene10 とCry j I との融合蛋白質 (T7Cry j I) を発現したBL21の抽出液、スギ花粉より精製した天然型Cry j I を同時にプロットして反応を調べた。図2に示すように、2人の患者血清がT7 Cry j II と反応した。2人の患者血清ともT7 Cry j II、天然型Cry j Iには反応しているがpGEMEX-1を保持するBL21抽出液およびT7 Cry j Iには反応していない。これらの結果からT7Cry j IIはスギ花粉症患者血清中のIgE と反応する抗原性を持っていることが確認された。

【0053】<オーバーラップペプチドの合成>オーバーラップペプチドの合成は、Peptide Synthesizer PSSM-8 (島津製作所製) を用いて行なった。配列番号2に示すCry j IIの一次構造を基にして、N末端側55番目のAla から始まり、C末端のPro まで、10残基のオーバーラップ部分を含む15量体のオーバーラップペプチド90種類を合成した。図3~6にアミノ酸の1文字コードを用いて、合成した全てのオーバーラップペプチドを示す。

【0054】<B細胞株の樹立>Ficoll-Paque比重遠心法で得た末梢血リンパ球 (1×10^6) を、約 1×10^6 PFU (plaque forming units) のEpstein-Barr virus (EBV) と共に37℃で1時間インキュベートし、ウイルスを細胞に感染させた。このウイルス感染細胞を24ウェル培養プレートに移し、100ng/mlのサイクロスポリンA の存在下で2週間前後培養すると、B細胞コロニーが出現してくる。この時点で半分に分け、新しいウェルに植え継いだ。順次この操作を繰り返して継代培養を行っていくと、自己増殖可能なB細胞が出現してくる場合がある。この自己増殖B細胞を含むウェルの細胞をイクスパンド (expand) し、増殖を確認した後、25cm² 培養フラスコに移して更に30~50日間培養を行い、EBVによってトランスフォームされた (EBV-transformed) B細胞株を得

た。B細胞株の一部は凍結保存した。

【0055】<Cry j II抗原特異的T細胞ラインの樹立>スギ花粉症患者18名末梢血からリンパ球を通常用いられているFicoll-paque比重遠心法で単離し、使用するまで液体窒素中に保存した。スギ花粉症患者の末梢血リンパ球 (4×10^6 個) を、2mlの自己の血漿20% を添加したRPMI-1640 に懸濁し、10μg/mlの大腸菌で発現させ精製した組換えCry j II抗原と共に24穴培養プレート上で7-8日間培養した。Cry j II抗原刺激を受けて活性化された (幼弱化反応、blastogenesis) T細胞が顕微鏡下で確認できた時点で5 Unit/ml のIL-2を添加し、一晚培養した。翌日からは、20 Unit/ml IL-2、20% ヒトAB型血清 (市販品) を添加したRPMI-1640 で毎日培養液を代えながら、9日間培養した。この時点で、Cry j II抗原を特異的に認識する増殖したT細胞ラインの一部を凍結保存した。さらにT細胞ラインを上記培養液中で4日間培養し、エピトープの同定用の細胞とした。

【0056】<T細胞エピトープを含むオーバーラップペプチドの同定>18名の花粉症患者から樹立したT細胞ラインについてそれぞれスギ花粉アレルゲンオーバーラップペプチドとともに培養し、Cry j II抗原特異的T細胞エピトープを含むペプチドの同定を行った。

【0057】T細胞ラインと同一の患者から樹立した培養B細胞株を50μg/mlのマイトマイシンCで30分間処理し、細胞をRPMI-1640で4回洗浄した。このB細胞を96穴平底プレート (96-well flat-bottomed plate) に播種 (5×10^4 /well) した後、Cry j II (25μg/ml最終濃度) あるいは各オーバーラップペプチド (最終濃度0.5 μM) を各々のウェルに添加し、約60~90分間培養した。T細胞ライン (2×10^4 /well) を各ウェルに播種し、48時間培養の後、0.5 μl/Ci [³H]チミジンをウェルに添加し、さらに16時間培養した。細胞を細胞ハーベスターを用いてガラスフィルター上に捕集し、乾燥してから、細胞内に取込まれた [³H]チミジンのカウント (cpm) を液体シンチレーションカウンターで測定した。

【0058】測定はtriplicate cultureで行い、結果は、オーバーラップペプチド刺激によるT細胞ラインの [³H]チミジン取込み量 (cpm) を、抗原を添加しない場合 (コントロール) の [³H]チミジン取込み量 (cpm) で割った値である刺激係数 (stimulation index; SI) で算出し、SIが2以上の値を示したオーバーラップペプチドを、T細胞エピトープを含むオーバーラップペプチドと同定した。図7及び図8は、18名の花粉症患者からそれぞれ樹立されたCry j II抗原特異的T細胞ラインの少なくとも1種類が認識する少なくとも一つのT細胞エピトープを含むペプチドを示す。また、図9は、全てのオーバーラップペプチドの「平均刺激係数」(複数の実験によって得られた刺激係数の平均値) 「出現頻度(%)」 「重要度指数」を示している。

17

【0059】

【発明の効果】本発明のCry j IIの少なくとも一つのエピトープを含むペプチド、T細胞エピトープを含むペプチドは、スギ花粉症の診断、予防及び治療に有用である。

【0060】さらにまた、HLA クラス 分子には結合するが、T細胞には情報が伝わらないようなアナログペプチドを合成し、これらのペプチドを競争阻害によるスギ

配列：

```

Met Ala Met Lys Leu Ile Ala Pro Met Ala Phe Leu Ala Met Gln Leu
      5              10              15
Ile Ile Met Ala Ala Ala Glu Asp Gln Ser Ala Gln Ile Met Leu Asp
      20              25              30
Ser Val Val Glu Lys Tyr Leu Arg Ser Asn Arg Ser Leu Arg Lys Val
      35              40              45
Glu His Ser Arg His Asp Ala Ile Asn Ile Phe Asn Val Glu Lys Tyr
      50              55              60
Gly Ala Val Gly Asp Gly Lys His Asp Cys Thr Glu Ala Phe Ser Thr
      65              70              75              80
Ala Trp Gln Ala Ala Cys Lys Asn Pro Ser Ala Met Leu Leu Val Pro
      85              90              95
Gly Ser Lys Lys Phe Val Val Asn Asn Leu Phe Phe Asn Gly Pro Cys
      100             105             110
Gln Pro His Phe Thr Phe Lys Val Asp Gly Ile Ile Ala Ala Tyr Gln
      115             120             125
Asn Pro Ala Ser Trp Lys Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys
      130             135             140
Leu Thr Gly Phe Thr Leu Met Gly Lys Gly Val Ile Asp Gly Gln Gly
      145             150             155             160
Lys Gln Trp Trp Ala Gly Gln Cys Lys Trp Val Asn Gly Arg Glu Ile
      165             170             175
Cys Asn Asp Arg Asp Arg Pro Thr Ala Ile Lys Phe Asp Phe Ser Thr
      180             185             190
Gly Leu Ile Ile Gln Gly Leu Lys Leu Met Asn Ser Pro Glu Phe His
      195             200             205
Leu Val Phe Gly Asn Cys Glu Gly Val Lys Ile Ile Gly Ile Ser Ile
      210             215             220
Thr Ala Pro Arg Asp Ser Pro Asn Thr Asp Gly Ile Asp Ile Phe Ala
      225             230             235             240
Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr Gly Asp Asp
      245             250             255
Cys Val Ala Ile Gly Thr Gly Ser Ser Asn Ile Val Ile Glu Asp Leu
      260             265             270
Ile Cys Gly Pro Gly His Gly Ile Ser Ile Gly Ser Leu Gly Arg Glu
      275             280             285
Asn Ser Arg Ala Glu Val Ser Tyr Val His Val Asn Gly Ala Lys Phe
      290             295             300
Ile Asp Thr Gln Asn Gly Leu Arg Ile Lys Thr Trp Gln Gly Gly Ser
      305             310             315             320
Gly Met Ala Ser His Ile Ile Tyr Glu Asn Val Glu Met Ile Asn Ser

```

18

花粉症発症予防に用いることも可能である。

【0061】

【配列表】

配列番号：1

配列の長さ：514

配列の型：アミノ酸

トポロジー：直鎖状

配列の種類：タンパク質

325 330 335
 Glu Asn Pro Ile Leu Ile Asn Gln Phe Tyr Cys Thr Ser Ala Ser Ala
 340 345 350
 Cys Gln Asn Gln Arg Ser Ala Val Gln Ile Gln Asp Val Thr Tyr Lys
 355 360 365
 Asn Ile Arg Gly Thr Ser Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys
 370 375 380
 Ser Asp Ser Met Pro Cys Lys Asp Ile Lys Leu Ser Asp Ile Ser Leu
 385 390 395 400
 Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn Ala Asn
 405 410 415
 Gly Tyr Phe Ser Gly His Val Ile Pro Ala Cys Lys Asn Leu Ser Pro
 420 425 430
 Ser Ala Lys Arg Lys Glu Ser Lys Ser His Lys His Pro Lys Thr Val
 435 440 445
 Met Val Glu Asn Met Arg Ala Tyr Asp Lys Gly Asn Arg Thr Arg Ile
 450 455 460
 Leu Leu Gly Ser Arg Pro Pro Asn Cys Thr Asn Lys Cys His Gly Cys
 465 470 475 480
 Ser Pro Cys Lys Ala Lys Leu Val Ile Val His Arg Ile Met Pro Gln
 485 490 495
 Glu Tyr Tyr Pro Gln Arg Trp Ile Cys Ser Cys His Gly Lys Ile Tyr
 500 505 510
 His Pro

配列番号: 2

配列の長さ: 460

配列の型: アミノ酸

トポロジー: 直鎖状

配列の種類: タンパク質

配列:

Ala Ile Asn Ile Phe Asn Val Glu Lys Tyr Gly Ala Val Gly Asp Gly
 5 10 15
 Lys His Asp Cys Thr Glu Ala Phe Ser Thr Ala Trp Gln Ala Ala Cys
 20 25 30
 Lys Asn Pro Ser Ala Met Leu Leu Val Pro Gly Ser Lys Lys Phe Val
 35 40 45
 Val Asn Asn Leu Phe Phe Asn Gly Pro Cys Gln Pro His Phe Thr Phe
 50 55 60
 Lys Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser Trp Lys
 65 70 75 80
 Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu
 85 90 95
 Met Gly Lys Gly Val Ile Asp Gly Gln Gly Lys Gln Trp Trp Ala Gly
 100 105 110
 Gln Cys Lys Trp Val Asn Gly Arg Glu Ile Cys Asn Asp Arg Asp Arg
 115 120 125
 Pro Thr Ala Ile Lys Phe Asp Phe Ser Thr Gly Leu Ile Ile Gln Gly
 130 135 140
 Leu Lys Leu Met Asn Ser Pro Glu Phe His Leu Val Phe Gly Asn Cys
 145 150 155 160
 Glu Gly Val Lys Ile Ile Gly Ile Ser Ile Thr Ala Pro Arg Asp Ser
 165 170 175

21
 Pro Asn Thr Asp Gly Ile Asp Ile Phe Ala Ser Lys Asn Phe His Leu
 180 185 190
 Gln Lys Asn Thr Ile Gly Thr Gly Asp Asp Cys Val Ala Ile Gly Thr
 195 200 205
 Gly Ser Ser Asn Ile Val Ile Glu Asp Leu Ile Cys Gly Pro Gly His
 210 215 220
 Gly Ile Ser Ile Gly Ser Leu Gly Arg Glu Asn Ser Arg Ala Glu Val
 225 230 235 240
 Ser Tyr Val His Val Asn Gly Ala Lys Phe Ile Asp Thr Gln Asn Gly
 245 250 255
 Leu Arg Ile Lys Thr Trp Gln Gly Gly Ser Gly Met Ala Ser His Ile
 260 265 270
 Ile Tyr Glu Asn Val Glu Met Ile Asn Ser Glu Asn Pro Ile Leu Ile
 275 280 285
 Asn Gln Phe Tyr Cys Thr Ser Ala Ser Ala Cys Gln Asn Gln Arg Ser
 290 295 300
 Ala Val Gln Ile Gln Asp Val Thr Tyr Lys Asn Ile Arg Gly Thr Ser
 305 310 315 320
 Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys Ser Asp Ser Met Pro Cys
 325 330 335
 Lys Asp Ile Lys Leu Ser Asp Ile Ser Leu Lys Leu Thr Ser Gly Lys
 340 345 350
 Ile Ala Ser Cys Leu Asn Asp Asn Ala Asn Gly Tyr Phe Ser Gly His
 355 360 365
 Val Ile Pro Ala Cys Lys Asn Leu Ser Pro Ser Ala Lys Arg Lys Glu
 370 375 380
 Ser Lys Ser His Lys His Pro Lys Thr Val Met Val Glu Asn Met Arg
 385 390 395 400
 Ala Tyr Asp Lys Gly Asn Arg Thr Arg Ile Leu Leu Gly Ser Arg Pro
 405 410 415
 Pro Asn Cys Thr Asn Lys Cys His Gly Cys Ser Pro Cys Lys Ala Lys
 420 425 430
 Leu Val Ile Val His Arg Ile Met Pro Gln Glu Tyr Tyr Pro Gln Arg
 435 440 445
 Trp Ile Cys Ser Cys His Gly Lys Ile Tyr His Pro
 450 455 460

配列番号 : 3

配列の長さ : 1542

配列の型 : 核酸

トポロジー : 直鎖状

配列の種類 : cDNA to mRNA

配列 :

ATGCCCATGA AATTAATTGC TCCAATGGCC TTTCTGGCCA TGCAATTGAT TATAATGGCG 60
 GCAGCAGAAG ATCAATCTGC CCAAATTATG TTGGACAGTG TTGTCGAAAA ATATCTTAGA 120
 TCGAATCGGA GTTAAAGAAA AGTTGAGCAT TCTCGTCATG ATGCTATCAA CATCTTCAAT 180
 GTGGAAAAAT ATGGCGCAGT AGGCGATGGA AAGCATGATT GCACTGAGGC ATTTTCAACA 240
 GCATGGCAAG CTGCATGCAA AAACCATCA GCAATGTTGC TTGTGCCAGG CAGCAAGAAA 300
 TTTGTTGTAA ACAATTTGTT CTTCATGGG CCATGTCAAC CTCACTTTAC TTTTAAGGTA 360
 GATGGGATAA TAGCTGCGTA CCAAAATCCA GCGAGCTGGA AGAATAATAG AATATGGTTG 420
 CAGTTTGCTA AACTTACAGG TTTTACTCTA ATGGGTAAAG GTGTAATTGA TGGGCAAGGA 480
 AAACAATGGT GGGCTGGCCA ATGTAAATGG GTCAATGGAC GAGAAATTG CAACGATCGT 540
 GATAGACCAA CAGCCATTAA ATTCGATTTT TCCACGGGTC TGATAATCCA AGGACTGAAA 600

23

24

CTAATGAACA GTCCCGAATT TCATTTAGTT TTTGGGAATT GTGAGGGAGT AAAAATCATC 660
 GGCATTAGTA TTACGGCACC GAGAGACAGT CCTAACACTG ATGGAATTGA TATCTTTGCA 720
 TCTAAAACT TTCACTTACA AAAGAACAG ATAGGAACAG GGGATGACTG CGTCGCTATA 780
 GGCACAGGGT CTTCTAATAT TGTGATTGAG GATCTGATTT GCGGTCCAGG CCATGGAATA 840
 AGTATAGGAA GTCTTGGGAG GGAAAACTCT AGAGCAGAGG TTTCATACGT GCACGTAAAT 900
 GGGGCTAAAT TCATAGACAC ACAAATGGA TTAAGAATCA AAACATGGCA GGGTGGTTCA 960
 GGCATGGCAA GCCATATAAT TTATGAGAAT GTTGAAATGA TAAATTCGGA GAACCCCAT 1020
 TTAATAATC AATTCTACTG CACTTCGGCT TCTGCTTGCC AAAACCAGAG GTCTCGGTT 1080
 CAAATCCAAG ATGTGACATA CAAGAACATA CGTGGGACAT CAGCAACAGC AGCAGCAATT 1140
 CAACTTAAGT GTAGTGACAG TATGCCCTGC AAAGATATAA AGCTAAGTGA TATATCTTTG 1200
 AAGCTTACCT CAGGAAAAAT TGCTTCCTGC CTTAATGATA ATGCAATGG ATATTTACGT 1260
 GGACACGTCA TCCCTGCATG CAAGAATTGA AGTCCAAGT CTAAGCGAAA AGAATCTAAA 1320
 TCCCATAAAC ACCCAAAAC TGTAAATGTT GAAAATATGC GAGCATATGA CAAGGGTAAC 1380
 AGAACACGCA TATTGTTGGG GTCGAGGCCT CCGAATTGTA CAAACAAATG TCATGGTTGC 1440
 AGTCCATGTA AGGCCAAGTT AGTTATTGTT CATCGTATTA TGCCGCAGGA GTATTATCCT 1500
 CAGAGGTGGA TATGCAGCTG TCATGGCAAA ATCTACCATC CA 1542

配列番号 : 4

配列の長さ : 1380

配列の型 : 核酸

* トポロジー : 直鎖状

配列の種類 : cDNA to mRNA

*

配列 :

GCTATCAACA TCTTCAATGT GGAAAAATAT GGCGCAGTAG GCGATGGAAA GCATGATTGC 60
 ACTGAGGCAT TTCAACAGC ATGGCAAGCT GCATGCAAAA ACCCATCAGC AATGTTGCTT 120
 GTGCCAGGCA GCAAGAAAT TGTGTAAAC AATTTGTTCT TCAATGGGCC ATGTCAACCT 180
 CACTTTACTT TTAAGGTAGA TGGGATAATA GCTGCGTACC AAAATCCAGC GAGCTGGAAG 240
 AATAATAGAA TATGGTTGCA GTTTGCTAAA CTTACAGGTT TTAATCTAAT GGGTAAAGGT 300
 GTAATTGATG GGCAAGGAAA ACAATGGTGG GCTGGCCAAT GTAAATGGGT CAATGGACGA 360
 GAAATTTGCA ACGATCGTA TAGACCAACA GCCATTAAAT TCGATTTTTC CACGGGTCTG 420
 ATAATCCAAG GACTGAACT AATGAACAGT CCCGAATTTT ATTTAGTTTT TGGGAATTGT 480
 GAGGGAGTAA AAATCATCGG CATTAGTATT ACGGCACCGA GAGACAGTCC TAACACTGAT 540
 GGAATTGATA TCTTTGCATC TAAAACTTT CACTTACAAA AGAACACGAT AGGAACAGGG 600
 GATGACTGCG TCGCTATAGG CACAGGGTCT TCTAATATTG TGATTGAGGA TCTGATTGTC 660
 GGTCCAGGCC ATGGAATAAG TATAGGAAGT CTTGGGAGGG AAAACTCTAG AGCAGAGGTT 720
 TCATACGTGC ACGTAAATGG GGCTAAATTC ATAGACACAC AAAATGGATT AAGAATCAAA 780
 ACATGGCAGG GTGGTTCAGG CATGGCAAGC CATATAATTT ATGAGAATGT TGAAATGATA 840
 AATTCGAGA ACCCATATT AATAAATCAA TTCTACTGCA CTTGGGCTTC TGCTTGCCAA 900
 AACCAGAGGT CTGCGGTTCA AATCCAAGAT GTGACATACA AGAACATACG TGGGACATCA 960
 GCAACAGCAG CAGCAATTCA ACTTAAGTGT AGTGACAGTA TGCCCTGCAA AGATATAAAG 1020
 CTAAGTGATA TATCTTTGAA GCTTACCTCA GGGAAAAATTG CTTCTGCCT TAATGATAAT 1080
 GCAAATGGAT ATTTCACTGG ACACGTCATC CCTGCATGCA AGAATTTAAG TCCAAGTGCT 1140
 AAGCGAAAAG AATCTAAATC CCATAAACAC CCAAAAACTG TAATGGTTGA AAATATGCGA 1200
 GCATATGACA AGGGTAACAG AACACGCATA TTGTTGGGGT CGAGGCCTCC GAATTGTACA 1260
 AACAAATGTC ATGGTTGCAG TCCATGTAAG GCCAAGTTAG TTATTGTTCA TCGTATTATG 1320
 CCGCAGGAGT ATTATCCTCA GAGGTGGATA TGCAGCTGTC ATGGCAAAAT CTACCATCCA 1380

配列番号 : 5

配列の長さ : 1733

配列の型 : 核酸

トポロジー : 直鎖状

配列の種類 : cDNA to mRNA

配列 :

AGTTGAGTTC GAGACAAGTA TAGAAGAAT TTTCTTTTAT TAAATGGCC ATGAAATTAA 60
 TTGCTCCAAT GGCTTTCTG GCCATGCAAT TGATTATAAT GGCGGCAGCA GAAGATCAAT 120
 CTGCCCCAAT TATGTTGGAC AGTGTGTGCG AAAAATATCT TAGATCGAAT CGGAGTTTAA 180

25

26

GAAAAGTTGA GCATTCTCGT CATGATGCTA TCAACATCTT CAATGTGGAA AAATATGGCG 240
 CAGTAGGCGA TGGAAAGCAT GATTGCACTG AGGCATTTTC AACAGCATGG CAAGCTGCAT 300
 GCAAAAACCC ATCAGCAATG TTGCTTGTGC CAGGCAGCAA GAAATTTGTT GTAAACAATT 360
 TGTCTTCAA TGGGCCATGT CAACCTCACT TTAATTTTAA GGTAGATGGG ATAATAGCTG 420
 CGTACCAAAA TCCAGCGAGC TGAAGAATA ATAGAATATG GTTGCACTTT GCTAAACTTA 480
 CAGGTTTTAC TCTAATGGGT AAAGGTGTAA TTGATGGGCA AGGAAAACAA TGGTGGGCTG 540
 GCCAATGTAA ATGGGTCAAT GGACGAGAAA TTTGCAACGA TCGTGATAGA CCAACAGCCA 600
 TTAAATTCGA TTTTCCACG GGTCTGATAA TCCAAGGACT GAAACTAATG AACAGTCCCG 660
 AATTTTCATT AGTTTTTGGG AATTGTGAGG GAGTAAAAAT CATCGGCATT AGTATTACGG 720
 CACCGAGAGA CAGTCCTAAC ACTGATGGAA TTGATATCTT TGCATCTAAA AACTTTCACT 780
 TACAAAAGAA CACGATAGGA ACAGGGGATG ACTGCGTCGC TATAGGCACA GGTCTTCTA 840
 ATATTGTGAT TGAGGATCTG ATTTGCGGTC CAGGCCATGG AATAAGTATA GGAAGTCTTG 900
 GGAGGGAAAA CTCTAGAGCA GAGGTTTCAT ACGTGCACGT AAATGGGGCT AAATTCATAG 960
 ACACACAAAA TGGATTAAGA ATCAAAACAT GGCAGGGTGG TTCAGGCATG GCAAGCCATA 1020
 TAATTTATGA GAATGTTGAA ATGATAAATT CGGAGAACCC CATATTAATA AATCAATTCT 1080
 ACTGCACTTC GGCTTCTGCT TGCCAAAACC AGAGGTCTGC GGTTCAAATC CAAGATGTGA 1140
 CATACAAGAA CATACGTGGG ACATCAGCAA CAGCAGCAGC AATTCAACTT AAGTGTAGTG 1200
 ACAGTATGCC CTGCAAAGAT ATAAAGCTAA GTGATATATC TTTGAAGCIT ACCTCAGGGA 1260
 AAATTGCTTC CTGCCTTAAT GATAATGCAA ATGGATATTT CAGTGGACAC GTCATCCCTG 1320
 CATGCAAGAA TTTAAGTCCA AGTGCTAAGC GAAAAGAATC TAAATCCCAT AAACACCCAA 1380
 AAATCTGAAT GGTGAAAAAT ATGCGAGCAT ATGACAAGGG TAACAGAACA CGCATATTGT 1440
 TGGGGTCGAG GCCTCCGAAT TGTACAAACA AATGTCATGG TTGCAGTCCA TGTAAAGGCCA 1500
 AGTTAGTTAT TGTTATCGT ATTATGCCGC AGGAGTATTA TCCTCAGAGG TGGATATGCA 1560
 CCTGTCATGG CAAATCTAC CATCCATAAT GAGATACATT GAAACTGTAT GTGCTAGTGA 1620
 ATATTCTTGT GGTACAATAT TAGAACTGAT ATTGAAAAATA AATCATCAAT GTTTCTAAGG 1680
 CATTTATAAT AGATTATATT AATGGTTCAA AAAAAAAAAA AAAAAAAAAA AAA 1733

【図面の簡単な説明】

【図1】スギ花粉アレルゲンCry j IIのN末端から10残基のアミノ酸配列(A)。スギ花粉アレルゲンCry j IIのN末端から10残基のアミノ酸配列から予想されるDNA配列(B)。スギ花粉アレルゲンCry j IIをコードするcDNAをスクリーニングするためのプローブのDNA配列(C)。

【図2】T7 Cry j IIの抗原性を、2名のスギ花粉症患者の血清を用いて、ウェスタンブロット法により同定した結果を示す。レーン1はpMGEMEX-1（陰性対照）を保持するBL21(DE3)、レーン2はT7 Cry j Iを発現したBL21(DE3)、レーン3はT7Cry j IIを発現したBL21(DE3)、レーン4はスギ花粉より精製したCry j Iをそれぞれ示す。A、Bは血清の由来する患者が異なるのみで、他は同じである。

【図3】Cry j IIの全アミノ酸配列をカバーするオーバーラップペプチドを示す。

【図4】Cry j IIの全アミノ酸配列をカバーするオーバーラップペプチドを示す。

【図5】Cry j IIの全アミノ酸配列をカバーするオーバーラップペプチドを示す。

【図6】Cry j IIの全アミノ酸配列をカバーするオーバーラップペプチドを示す。

【図7】Cry j IIの少なくとも一つのT細胞エピトープを含むペプチドを示す。

【図8】Cry j IIの少なくとも一つのT細胞エピトープを含むペプチドを示す。

【図9】18名のスギ花粉症患者から樹立されたCry j IIアレルゲンに特異的なT細胞ラインがそれぞれ認識するオーバーラップペプチドの重要度指数を示す。

【図6】

86. (426-440) C S P C K A K L V I V H R I M
 87. (431-445) A X L V I V H R I M P Q E Y Y
 88. (436-450) V H R I M P Q E Y Y P Q R W I
 89. (441-455) P Q E Y Y P Q R W I C S C H G
 90. (446-460) P Q R W I C S C H G K I Y H P

【図1】

(A) アミノ酸配列 Ala Ile Asn Ile Phe Asp Val Glu Lys Tyr

(B) 予想されるcDNA配列 5' GCT ATT AAT ATT TTT AAT GTT GAA AAA TAT
 5' GCC ATC AAC ATC TTC AAC GTC GAG AAG TAC
 5' GCA ATA AAT ATA TTT AAT GTA GAA AAA TAT
 5' GCG ATT AAT ATT TTT AAT GTC GAA AAA TAT

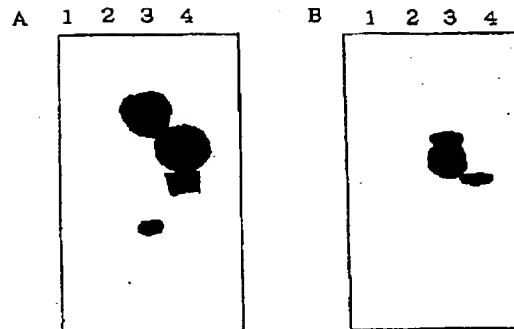
(C) 合成したプローブ 3' CGG TAG TTG TAG AAG TTG CAG CTT TTT ATG
 3' CGT TAT TTG TAT AAG TTG CAC CTT TTT ATG

【図3】

1. (1-15) A I N I F N V E K Y G A V G D
 2. (6-20) N V E K Y G A V G D G K H D C
 3. (11-25) G A V G D G K H D C T E A F S
 4. (16-30) G K H D C T E A F S T A W Q A
 5. (21-35) T E A F S T A W Q A A C K N P
 6. (26-40) T A W Q A A C K N P S A M L L
 7. (31-45) A C K N P S A M L L V P G S K
 8. (38-50) S A M L L V P G S K K F V V N
 9. (41-55) V P G S K K F V V N N L F F N
 10. (46-60) K F V V N N L F F N G P C Q P
 11. (51-65) N L F F N G P C Q P H F T F K
 12. (56-70) G P C Q P H F T F K V D G I I
 13. (61-75) H F T F K V D G I I A A Y Q N
 14. (66-80) V D G I I A A Y Q N P A S W K
 15. (71-85) A A Y Q N P A S W K N N R I H
 16. (76-90) P A S W K N N R I W L Q F A K
 17. (81-95) N N R I W L Q F A K L T G F T
 18. (86-100) L Q F A K L T G F T L M G K G
 19. (91-105) L T G F T L M G K G V I D G Q
 20. (96-110) L M G K G V I D G Q G K Q W W
 21. (101-115) V I D G Q G K Q W W A G Q C K
 22. (106-120) G K Q W W A G Q C K W Y N G B
 23. (111-125) A G Q C K W Y N G B E I C N D
 24. (116-130) W Y N G B E I C N D E D R P T
 25. (121-135) E I C N D R D B P T A I K F D
 26. (126-140) R D R P T A I K F D F S T G L
 27. (131-145) A I K F D F S T G L I I Q G L

28. (136-150) F S T G L I I Q G L K L M N S
 29. (141-155) I I Q G L K L M N S P E F H L
 30. (146-160) K L M N S P E F H L V F G N C
 31. (151-165) P E F H L V F G N C E G V K I
 32. (156-170) V F G N C E G V K I I G I S I
 33. (161-175) E G V K I I G I S I T A P R D
 34. (166-180) I G I S I T A P R D S P N T D
 35. (171-185) T A P R D S P N T D G I D I F
 36. (176-190) S P N T D G I D I F A S K N F
 37. (181-195) G I D I F A S K N F H L Q K N
 38. (186-200) A S K N F H L Q K N T I G T G
 39. (191-205) H L Q K N T I G T G D D C V A
 40. (196-210) T I G T G D D C V A I G T G S
 41. (201-215) D D C V A I G T G S S N I V I
 42. (206-220) I G T G S S N I V I E D L I C
 43. (211-225) S N I V I E D L I C G P G H G
 44. (216-230) E D L I C G P G H G I S I G S
 45. (221-235) G P G H G I S I G S L G R E N
 46. (226-240) I S I G S L G R E N S R A E V
 47. (231-245) L G R E N S R A E V S Y V H V
 48. (236-250) S R A E V S Y V H V N G A K F
 49. (241-255) S Y V H V N G A K F I D T Q N
 50. (246-260) N G A K F I D T Q N G L R I K
 51. (251-265) I D T Q N G L R I K T W Q G G
 52. (256-270) G L R I K T W Q G G S G M A S
 53. (261-275) T W Q G G S G M A S H I I Y E
 54. (266-280) S G M A S H I I Y E N V E M I
 55. (271-285) H I I Y E N V E M I N S E N P
 56. (276-290) N V E M I N S E N P I L I N Q
 57. (281-295) N S E N P I L I N Q F Y C T S
 58. (286-300) I L I N Q F Y C T S A S A C Q
 59. (291-305) F Y C T S A S A C Q N Q R S A
 60. (296-310) A S A C Q N Q R S A V Q I Q D
 61. (301-315) N Q R S A V Q I Q D V T Y K N
 62. (306-320) V Q I Q D V T Y K N I R G T S
 63. (311-325) V T Y K N I R G T S A T A A A
 64. (316-330) I R G T S A T A A A I Q L K C
 65. (321-335) A T A A A I Q L K C S D S M P
 66. (326-340) I Q L K C S D S M P C K D I K
 67. (331-345) S D S M P C K D I K L S D I S
 68. (336-350) C K D I K L S D I S L K L T S
 69. (341-355) L S D I S L K L T S O K I A S
 70. (346-360) L K L T S G K I A S C L N D N
 71. (351-365) G K I A S C L N D N A N G Y F
 72. (356-370) C L N D N A N G Y F S G H V I
 73. (361-375) A N G Y F S G H V I P A C K N
 74. (366-380) S G H V I P A C K N L S P S A
 75. (371-385) P A C K N L S P S A K R K E S
 76. (376-390) L S P S A K R K E S K S B K H
 77. (381-395) K R K E S K S H K H P K T V M
 78. (386-400) K S H K H P K T V M V E N M R
 79. (391-405) P K T V M V E N M R A Y D K G
 80. (396-410) V E N M R A Y D K G N R T R I
 81. (401-415) A Y D K G N R T R I L L G S R
 82. (406-420) N R T R I L L G S R P P N C T
 83. (411-425) L L G S R P P N C T N K C H G
 84. (416-430) P P N C T N K C H G C S P C K
 85. (421-435) N E C H G C S P C K A K L V I

【図2】



1. pGEMEX-1を保持するBL-21
2. 17-Cryj1を発現したBL-21
3. 17-Cryj1を発現したBL-21
4. スギ花粉より精製したCryj1

【図4】

【図5】

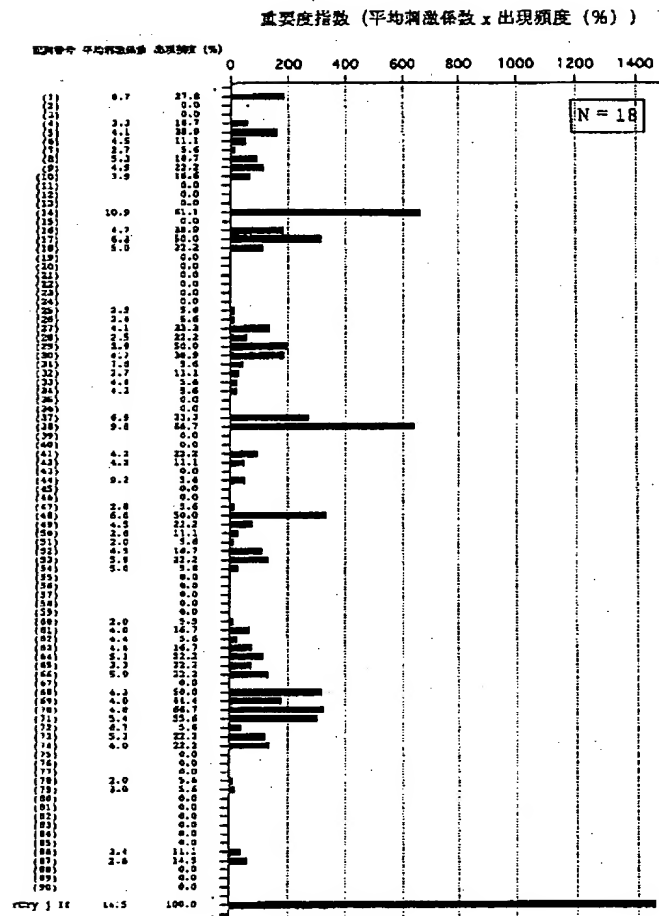
【図7】

1. Ala Ile Asn Ile Phe Asn Val Glu Lys Tyr Gly Ala Val Gly Asp
 4. Gly Lys His Asp Cys Thr Glu Ala Phe Ser Thr Ala Trp Gln Ala
 5. Thr Glu Ala Phe Ser Thr Ala Trp Gln Ala Ala Cys Lys Asn Pro
 6. Thr Ala Trp Gln Ala Ala Cys Lys Asn Pro Ser Ala Met Leu Leu
 7. Ala Cys Lys Asn Pro Ser Ala Met Leu Leu Val Pro Gly Ser Lys
 8. Ser Ala Met Leu Leu Val Pro Gly Ser Lys Lys Phe Val Val Asn
 9. Val Pro Gly Ser Lys Lys Phe Val Val Asn Asn Leu Phe Phe Asn
 10. Lys Phe Val Val Asn Asn Leu Phe Phe Asn Gly Pro Cys Gln Pro
 14. Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser Trp Lys
 16. Pro Ala Ser Trp Lys Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys
 17. Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr
 18. Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu Met Gly Lys Gly
 26. Arg Asp Arg Pro Thr Ala Ile Lys Phe Asp Phe Ser Thr Gly Leu
 27. Ala Ile Lys Phe Asp Phe Ser Thr Gly Leu Ile Ile Gln Gly Leu
 28. Phe Ser Thr Gly Leu Ile Ile Gln Gly Leu Lys Leu Met Asn Ser
 29. Ile Ile Gln Gly Leu Lys Leu Met Asn Ser Pro Glu Phe His Leu
 30. Lys Leu Met Asn Ser Pro Glu Phe His Leu Val Phe Gly Asn Cys
 31. Pro Glu Phe His Leu Val Phe Gly Asn Cys Glu Gly Val Lys Ile
 32. Val Phe Gly Asn Cys Glu Gly Val Lys Ile Ile Gly Ile Ser Ile
 37. Gly Ile Asp Ile Phe Ala Ser Lys Asn Phe His Leu Gln Lys Asn
 38. Ala Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr Gly
 41. Asp Asp Cys Val Ala Ile Gly Thr Gly Ser Ser Asn Ile Val Ile
 42. Ile Gly Thr Gly Ser Ser Asn Ile Val Ile Glu Asp Leu Ile Cys
 44. Glu Asp Leu Ile Cys Gly Pro Gly His Gly Ile Ser Ile Gly Ser
 47. Leu Gly Arg Glu Asn Ser Arg Ala Glu Val Ser Tyr Val His Val
 48. Ser Arg Ala Glu Val Ser Tyr Val His Val Asn Gly Ala Lys Phe
 49. Ser Tyr Val His Val Asn Gly Ala Lys Phe Ile Asp Thr Gln Asn
 50. Asn Gly Ala Lys Phe Ile Asp Thr Gln Asn Gly Leu Arg Ile Lys

【図8】

51. Ile Asp Thr Gln Asn Gly Leu Arg Ile Lys Thr Trp Gln Gly Gly
 52. Gly Leu Arg Ile Lys Thr Trp Gln Gly Gly Ser Gly Met Ala Ser
 53. Thr Trp Gln Gly Gly Ser Gly Met Ala Ser His Ile Ile Tyr Glu
 54. Ser Gly Met Ala Ser His Ile Ile Tyr Glu Asn Val Glu Met Ile
 60. Ala Ser Ala Cys Gln Asn Gln Arg Ser Ala Val Gln Ile Gln Asp
 61. Asn Gln Arg Ser Ala Val Gln Ile Gln Asp Val Thr Tyr Lys Asn
 62. Val Gln Ile Gln Asp Val Thr Tyr Lys Asn Ile Arg Gly Thr Ser
 63. Val Thr Tyr Lys Asn Ile Arg Gly Thr Ser Ala Thr Ala Ala Ala
 64. Ile Arg Gly Thr Ser Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys
 65. Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys Ser Asp Ser Met Pro
 66. Ile Gln Leu Lys Cys Ser Asp Ser Met Pro Cys Lys Asp Ile Lys
 68. Cys Lys Asp Ile Lys Leu Ser Asp Ile Ser Leu Lys Leu Thr Ser
 69. Leu Ser Asp Ile Ser Leu Lys Leu Thr Ser Gly Lys Ile Ala Ser
 70. Leu Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys leu Asn Asp Asn
 71. Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn Ala Asn Gly Tyr Phe
 72. Cys Leu Asn Asp Asn Ala Asn Gly Tyr Phe Ser Gly His Val Ile
 73. Ala Asn Gly Tyr Phe Ser Gly His Val Ile Pro Ala Cys Lys Asn
 74. Ser Gly His Val Ile Pro Ala Cys Lys Asn Leu Ser Pro Ser Ala
 78. Lys Ser His Lys His Pro Lys Thr Val Met Val Glu Asn Met Arg
 79. Pro Lys Thr Val Met Val Glu Asn Met Arg Ala Tyr Asp Lys Gly
 86. Cys Ser Pro Cys Lys Ala Lys Leu Val Ile Val His Arg Ile Met
 87. Ala Lys Leu Val Ile Val His Arg Ile Met Pro Gln Glu Tyr Tyr

【図9】



フロントページの続き

(51) Int. Cl. 6

C 1 2 Q 1/68

G 0 1 N 33/53

識別記号

庁内整理番号

A 9453-4B

Q

F I

技術表示箇所

PATENT ABSTRACTS OF JAPAN

(11)Publication number : 08-047392

(43)Date of publication of application : 20.02.1996

(51)Int.Cl.

C12N 15/09

C07K 7/08

C07K 14/415

// A61K 39/36

C12Q 1/68

G01N 33/53

(21)Application number : 06-297840

(71)Applicant : MEIJI MILK PROD CO LTD

(22)Date of filing : 07.11.1994

(72)Inventor : SONE TOSHIO
KOMIYAMA NAOKI
KII KOUSUKE

(30)Priority

Priority number : 05276773
06134868

Priority date : 05.11.1993
26.05.1994

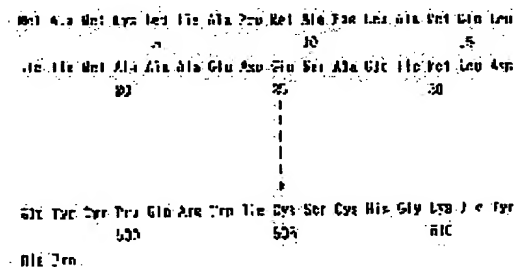
Priority country : JP
JP

(54) ALLERGEN CRY J II EPTOPE OF POLLEN OF JAPANESE CEDAR

(57)Abstract:

PURPOSE: To obtain a new protein or peptide useful for diagnosing, preventing and treating pollinosis of Japanese cedar, containing a specific amino acid sequence and having at least one epitope of allergen of pollen of Japanese cedar, especially T cell epitope.

CONSTITUTION: This protein or peptide contains the whole or part of an amino acid sequence including an amino acid sequence of the formula, has at least one epitope of allergen Cry-j-II of Japanese cedar, especially T cell epitope and is useful for diagnosing, preventing and treating pollinosis of Japanese cedar. The protein or peptide is obtained by passing whole RNA extracted from pollen of Japanese cedar by a conventional procedure through an oligo dT cellulose column to isolate mRNA, preparing a cDNA library using the mRNA, screening the library to select a cDNA coding for Cry-j-II, then incorporating the cDNA into a manifest station vector, transforming a host cell such as Escherichia coli and manifesting its gene.



LEGAL STATUS

[Date of request for examination] 06.11.2001

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

Copyright (C); 1998,2000 Japan Patent Office

*** NOTICES ***

Japan Patent Office is not responsible for any damages caused by the use of this translation.

1. This document has been translated by computer. So the translation may not reflect the original precisely.
2. **** shows the word which can not be translated.
3. In the drawings, any words are not translated.

CLAIMS

[Claim(s)]

[Claim 1] The protein or the peptide which holds at least one epitope including all or a part of amino acid sequences of array table 1 publication.

[Claim 2] DNA which carries out the code of protein or a peptide according to claim 1.

[Claim 3] DNA according to claim 2 characterized by including all or a part of base sequences of array table 3 publication.

[Claim 4] The protein or the peptide which holds at least one epitope including all or a part of amino acid sequences of array table 2 publication.

[Claim 5] DNA which carries out the code of protein or a peptide according to claim 4.

[Claim 6] DNA according to claim 5 characterized by including all or a part of base sequences of array table 4 publication.

[Claim 7] T The protein according to claim 1 or 4 or the peptide characterized by being a cell epitope.

[Claim 8] The protein according to claim 7 or the peptide characterized by including all or a part of each following amino acid sequence.

1. Ala Ile Asn Ile Phe Asn-Val-Glu-Lys-Tyr Gly-Ala-Val-Gly-Asp4. Gly-Lys-His-Asp-Cys Thr-Glu-Ala-Phe-Ser Thr-Ala-Trp-Gln-Ala5. Thr-Glu-Ala-Phe-Ser Thr-Ala-Trp-Gln-Ala Ala-Cys-Lys-Asn-Pro6. Thr-Ala-Trp-Gln-Ala Ala-Cys-Lys-Asn-Pro Ser Ala Met Leu Leu7. Ala Cys Lys Asn Pro Ser Ala Met Leu Leu Val Pro Gly Ser Lys8. Ser Ala Met Leu Leu Val Pro Gly Ser Lys Lys Phe Val Val Asn9. Val Pro Gly Ser Lys Lys Phe Val Val Asn Asn Leu Phe Phe Asn10. Lys Phe Val Val Asn Asn Leu Phe Phe Asn Gly Pro Cys Gln Pro14.Val Asp Gly Ile Ile Ala Ala Tyr Gln Asn Pro Ala Ser Trp Lys16.Pro Ala Ser Trp Lys Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys17.Asn Asn Arg Ile Trp Leu. Gln Phe Ala Lys Leu Thr Gly Phe Thr18.Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu Met Gly Lys Gly25.GluIle Cys Asn Asp Arg Asp Arg Pro Thr Ala Ile lys Phe Asp26.ArgAsp Arg Pro Thr Ala Ile Lys Phe Asp Phe Ser Thr Gly Leu27.Alalle Lys Phe Asp Phe Ser Thr Gly Leu Ile Ile Gln Gly Leu28.Phe Ser Thr Gly Leu Ile Ile Gln Gly Leu Lys Leu M t Asn Ser29.Ile IleGln Gly Leu Lys Leu Met Asn Ser Pro Glu Phe His Leu30.Lys LeuMet Asn Ser Pro Glu Phe His Leu Val Phe Gly Asn Cys31.Pro GluPhe His Leu Val Phe Gly Asn Cys Glu Gly Val Lys Ile32.Val Phe Gly Asn Cys Glu Gly Val Lys Ile Ile Gly Ile Ser Ile33.Glu Gly ValLys Ile Ile Gly Ile Ser Ile Thr Ala Pro Arg Asp34.Ile Gly IleSer Ile Thr Ala Pro Arg Asp Ser Pro Asn Thr Asp37.Gly Ile AspIle Phe Ala Ser Lys Asn Phe His Leu Gln Lys Asn38.Ala Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr Gly41.Asp Asp Cys ValAla Ile Gly Thr Gly Ser Ser Asn Ile Val Ile42. Ile Gly Thr GlySer Ser Asn Ile Val Ile Glu Asp Leu Ile Cys44.Glu Asp Leu IleCys Gly Pro Gly His Gly Il Ser Ile Gly Ser47.Leu Gly Arg Glu Asn Ser Arg Ala Glu Val Ser Tyr Val His Val48.Ser Arg Ala Glu ValSer Tyr Val His Val Asn Gly Ala Lys Phe49.Ser Tyr Val His Val AsnGly Ala Lys Phe Ile Asp Thr Gln Asn50.Asn Gly Ala Lys Phe Il AspThr Gln Asn Gly Leu Arg Ile Lys51. Ile Asp Thr Gln Asn Gly Leu Arg Ile Lys Thr Trp Gln Gly Gly52. Gly Leu Arg Ile Lys Thr Trp GlnGly Gly Ser Gly Met Ala Ser53.Thr Trp Gln Gly Gly S r Gly Met AlaSer His Ile Ile Tyr Glu54.Ser Gly Met Ala Ser His Ile Ile Tyr GluAsn Val Glu Met Ile60.Ala S r Ala Cys Gln Asn Gln

Arg Ser Ala Val Gln Ile Gln Asp61. Asn Gln Arg Ser Ala Val Gln Ile Gln Asp Val Thr Tyr Lys
 Asn62. Val Gln Ile Gln Asp Val Thr Tyr Lys Asn Ile Arg Gly Thr Ser63. Val Thr Tyr Lys Asn Ile Arg
 Gly Thr Ser Ala Thr Ala Ala Ala64. Ile Arg Gly Thr Ser Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys65.
 Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys Ser Asp Ser Met Pro66. Ile Gln Leu Lys Cys Ser Asp Ser
 Met Pro Cys Lys Asp Ile Lys68. Cys Lys Asp Ile Lys Leu Ser Asp Ile Ser L u Lys Leu Thr
 Ser69. Leu Ser Asp Ile Ser Leu Lys L u Thr Ser Gly Lys Ile Ala Ser70. Leu Lys Leu Thr Ser Gly
 Lys Ile Ala Ser Cys Leu Asn Asp Asn71. Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn Ala Asn Gly
 Tyr Phe72. Cys Leu Asn Asp Asn Ala Asn Gly Tyr Phe Ser Gly His Val Ile73. Ala Asn Gly Tyr Phe
 Ser Gly His Val Ile Pro Ala Cys Lys Asn74. Ser Gly His Val Ile Pro Ala Cys Lys Asn Leu Ser Pro
 Ser Ala78. Lys Ser His Lys His Pro Lys Thr Val Met Val Glu Asn Met Arg79. Pro Lys Thr Val Met
 Val Glu Asn Met Arg Ala Tyr Asp Lys Gly86. Cys Ser Pro Cys Lys Ala Lys Leu Val Ile Val His Arg
 Ile Met87. Ala Lys Leu Val Ile Val His Arg Ile Met Pro Gln Glu Tyr Tyr. [Claim 9] The protein
 according to claim 7 or the peptide characterized by including all or a part of each following
 amino acid sequence.

14. Val Asp Gly Ile Ile-Ala-Ala-Tyr-Gln Asn-Pro-Ala-Ser-Trp Lys17. Asn Asn Arg Ile
 Trp-Leu-Gln-Phe-Ala Lys-Leu-Thr-Gly-Phe Thr29. Ile Ile Gln Gly Leu-Lys-Leu-Met-Asn
 Ser-Pro-Glu-Phe-His Leu38. Ala Ser Lys Asn Phe-His-Leu-Gln-Lys Asn Thr Ile Gly Thr
 Gly48. Ser Arg Ala Glu Val Ser Tyr Val His Val Asn Gly Ala Lys Phe68. Cys Lys Asp Ile Lys Leu
 Ser Asp Ile Ser Leu Lys Leu Thr Ser70. Leu Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys Leu Asn
 Asp Asn71. Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn Ala Asn Gly Tyr Phe. [Claim 10] DNA
 which carries out the code of protein or a peptide according to claim 8 or 9.

[Claim 11] Protein or a peptide according to claim 8 or 9, the protein which has cross-reactivity
 immunologically, or a peptide.

[Claim 12] DNA which carries out the code of protein or a peptide according to claim 11.

[Translation done.]

* NOTICES *

Japan Patent Office is not responsible for any damages caused by the use of this translation.

1. This document has been translated by computer. So the translation may not reflect the original precisely.

2. **** shows the word which can not be translated.

3. In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Industrial Application] this invention is the epitope of Japan cedar pollen-allergen Cry j II useful for a diagnosis, prevention, or treatment of Japan cedar pollinosis, especially T. It is related with DNA which carries out the code of the protein containing a cell epitope, a peptide, this protein, or the peptide.

[0002]

[Description of the Prior Art] Japan cedar pollinosis is an allergic disease observed almost nationally at the beginning of spring Japan cedar pollen disperses, and presents the shape of an allergy accompanied by a sneeze, the itching of the pituita and an eye, etc. The number of patients is increasing rapidly in 1970 and afterwards, and is 10% of national [present] people. About 10 million people who hit weakness are troubled by Japan cedar pollinosis.

[0003] The allergic reaction which forms an allergic disease is R.G.H.Gell. R.R.A.Coombs I type - IV type four It is classified into the seed and Japan cedar pollinosis belongs to I type. The onset mechanism of I type allergy is as follows.

[0004] In the case of pollen, this allergen is a protein antigen although the molecule which triggers an allergic reaction is called allergen (it is also called an antigen on these specifications). If these visitor protein antigens trespass upon the inside of the body, it is incorporated by the antigen presenting cell (macrophage), and it will be decomposed by proteolytic enzyme, will become a peptide fragment, and will be shown on a cell membrane in the state where it combined with the major-histocompatibility-antigen complex (Major Histocompatibility Complex: major histocompatibility complex) class II molecule (Homo sapiens HLA class II molecule). HLA although a class II molecule shows polymorphism -- CD4+ the receptor of a T cell -- HLA the antigen peptide combined with the class II molecule -- the HLA the portion which shows the polymorphism of a class II molecule -- recognizing -- an antigen -- it is activated specifically Activated CD4+ A T cell is Th0. A cell, and Th1 / Th2 It specializes in a cell and various cytokines are produced. that time -- the cytokine production pattern of each cell -- differing -- **** -- Th1 IL-2 and IFN gamma -- Th2 IL-4, IL-5, and IL-10 etc. -- Th0 Both cytokine is produced.

[0005] On the other hand, it is B. A cell is macroglobulin to cell surface. Or immunoglobulin D It is expressing and is activated by incorporating an antigen in a cell. that time -- Th2 from -- B activated by the operation of the cytokine produced a cell -- an antibody forming cell -- specialization proliferation -- carrying out -- an antigen -- specific immunoglobulin E (immunoglobulin E) is produced Thus, produced immunoglobulin E It is immunoglobulin E to the basophilic leucocyte in a respiratory tract or the mast (obesity) cell under tunica-mucosa-nasi organization, or blood. It joins together firmly through a receptor and sensitization will be mat rialized.

[0006] Again, it is 1 when allergen tr spasses upon the inside of the body. The all rgen of a molecule is 2 on a mast cell or a basophilic leucocyt immediately. immunoglobulin E more than

a molecule It joins together and the structure of cross linkage is formed. Consequently, immunoglobulin E The receptors combined with the molecule meet, this serves as a trigger, how many kind thing enzyme in a cell membrane is activated, and various chemical transmitters, such as a histamine, a prostaglandin, and a leukotrien , are emitted from a cell. These chemical transmitters act on parts, such as tunica mucosa nasi and a respiratory tract, and cause the shape of a various allergy.

[0007] In addition, T It is T about the epitope recognized by the cell. A cell epitope and B It is B about the epitope recognized by the cell. It is called a cell epitope.

[0008] Since it is thought that the epitope of allergen is participating in an onset and exacerbation of I type allergy directly, it is useful for a diagnosis, prevention, and treatment of I type allergy to identify the epitope of allergen.

[0009] Isolation refining was done by **** and others and the main allergen of Japan cedar pollen was named Sugi Basic Protein (SBP) (Yasueda, H., et al., J.Allergy Clin.Immunol.71, 77-86, 1983). This SBP Molecular weight is 45-50kDa. WHO A nomenclature is followed and it is Present Cry j I. It is called. Furthermore, after that and Cry j I At the process of separation refining, it is Cryj I. The molecular weight from which antigenicity differs is 37kDa(s). Cry j II was separated (312 Taniai, M.et al.FEBS Letters 239, 329- 332, 1988, Sakaguchi, M.et al.Allergy 45,309- 1990).

[0010] It is Cry j I these results. Cry j II is Cry j I at a Japan cedar pollinosis patient, although it became clear that it is completely different protein. It was reported that both of Cry j II have reacted. Namely, 145 The inside of the Japan cedar pollinosis patient blood serum of a name, and 134 The blood serum of a name (92.4%) is Cry j I. And it reacts with Cry j II. 6 The blood serum of a name (4.1%) is Cry j I. It reacts and is 5. It became clear that the blood serum of a name (3.4%) reacts only with Cry j II (1993 43rd Japanese Society of Allergology per year, Hashimoto et al., the Japan Velerinary Medical Association size, ****, a national Sagamihara hospital, a wood primeval-izing **). That is, in the onset of Japan cedar pollinosis, it is Cry j I. And it was shown that Cry j II is both important.

[0011] Cry j I Cloning of the cDNA which carries out the code of it if it attaches is carried out, and it is based on the presumed amino acid sequence, and is T. The peptide containing a cell epitope is identified (WO 94/01560, "ALLERGENIC PROTEINS AND PEPTIDES FROM JAPANESE CEDAR POLLEN"). Cry j II -- N Ala of the amino acid sequence of an end, Ile, Asn, Ile, Phe, Asn, Val, Glu, and Lys And Tyr 10 amino acid residues are reported (Sakaguchi, M., et al., Allergy 45, 309-312, 1990) -- it is .

[0012]

[Problem(s) to be Solved by the Invention] this invention is at least one epitope, especially T of Japan cedar pollen-allergen Cry j II useful for a diagnosis, prevention, and treatment of Japan cedar pollinosis. It aims at offering DNA which carries out the code of the protein containing a cell epitope, a peptide, this protein, or the peptide.

[0013]

[Means for Solving the Problem] Invention-in-this-application persons are (1) Cry j II, in order to solve the above-mentioned technical problem. Elucidation Cry j II of all amino acid sequences (primary structure) (2) Production Cry j II of the overlap peptide which covers all amino acid sequences (3) T which recognizes allergen specifically It is establishment (4) according to an individual about a cell line. The overlap peptide containing the establishment (5) T-cell epitop of an antigen presenting cell (B cell strain) was identified, and this invention was completed. In addition, the epitope as used in the field of this invention is T. It is Following T although not restricted to a cell epitope. A cell epitop is explained in full detail. Each of these st ps are explained below.

[0014] (1) Cry j II Extraction RNA of cloning a.RNA of elucidation ** cDNA of all amino acid sequences In cas it extracts, protein is usually removed by the initial stage. For this r ason, th re is a method using prot in denaturation ag nts, such as the phenol extraction m thod, a

GUANIJUMU salt, a surfactant, and a urea, etc. as common practice.

[0015] RNA from Japan cedar pollen extraction — Breiteneder ** (Int.Arch.Allergy Appl.Immunol.87:19-24 and 1988) — improvement can be added and can be carried out to a method

[0016] Japan cedar pollen is suspended ten to 20 times in the extraction buffer solution (100mM LiCl, 10mMNa₂ EDTA, 1%SDS, 20% mercaptoethanol, and 100 mM Tris-HCl pH 9.0) of an amount, and size [of an equivalent phenol and the mixture (phenol : chloroform : isoamyl alcohol = 24:24:1) of chloroform / HOMOJIE] is added and carried out to this. Subsequently, centrifugal (10,000g, 10 - 15 minutes) is carried out, and it separates into a phenol chloroform layer and the bilayer of a water layer. The protein which denaturalized at this time shifts to a phenol chloroform layer, and a nucleic acid shifts to a water layer. Impurities, such as protein which adds and shakes a phenol chloroform mixture to a water layer, and remains in the water layer, are made to shift to a phenol chloroform layer, and are removed. Such operation is repeated twice.

[0017] the obtained water layer to RNA for extracting — high-concentration LiCl (2-4M) or — if CH₃ COONa (3M) exists — DNA and protein — a supernatant liquid — remaining — RNA other than tRNA The property to precipitate is used. LiCl of 2 -4M of the amount of said is added to a water layer, and it is RNA. It is made to precipitate. Subsequently, this water layer is dissolved in water and it is 0.1-0.3. The cold ethanol (-20 **) of ** is added, and it is RNA. It is made to precipitate (ethanol precipitation). Subsequently, centrifugal (10,000g, 30 minutes) is carried out, precipitation is collected, and it dissolves in water, and is all RNA. Fractionation is obtained.

[0018] b. mRNA mRNA of the composition Cry j II of manufacture and cDNA is poly (A) to a three-dash terminal. Since it has a chain, mRNA is adsorbed by the oligo-dT-cellulose column (Clontech Laboratories Inc. company make, CA, USA) which combined the deoxythymidine (dT) of 12 to 18 base as a ligand which carries out the complementation to this. Japan cedar pollen RNA The buffer solution (3M NaCl, 1mM EDTA, 10 mM Tris-HCl, pH 7.4) is added, and mRNA is made to stick to a column. mRNA is 2-3 of bed volume. It is eluted with the buffer solution (1mM EDTA, 10 mM Tris-HCl, pH 7.4) which does not contain NaCl of the amount of double.

[0019] Production of the cDNA library from obtained mRNA can perform the phage marketed now using the cDNA library production kit (Amersham International plc. company make, Buckinghamshare, England) used for the vector.

[0020] c. N of the screening Cry j II of Cry j II cDNA Synthetic DNA which has the base sequence presumed from this amino acid sequence although end amino acid 10 residue has already become clear It considers as a probe and is Cry j II cDNA. It can screen. DNA used for a probe When compounding, it is more desirable to design an oligonucleotide which is hybridized to two or more possible codon arrays rather than compounding all the oligonucleotides containing a possible codon. The indicator of the five prime end of this synthetic oligo NUKUREOCHIDOPU lobe is carried out to [gamma-32P] ATP by the polynucleotide kinase, and an electropositive clone is screened from the aforementioned cDNA library by the plaque hybridization method.

[0021] the obtained electropositive clone — phage DNA preparing — an insertion cDNA fragment — dissociating — pUC18 etc. — a sub clone is carried out to a plasmid An oligonucleotide primer is compounded if needed, a base sequence is determined by the Sanger method etc., and a clone is identified. Cry j II cDNA which this invention persons isolated The base sequence of an overall length is shown in the array number 5.

[0022] cDNA which carries out the code of Cry j II consists of 1733bp(s) on the whole, contains the open reading frame from the codon (nucleotide ATG of the 45-47th place) assumed to be initiation to a stop codon (nucleotide TAA of the 1587-1589th place), and is 514. The code of the amino acid is carried out. The amino acid sequence in which shows the base sequence of an open-reading-frame portion to the array number 3, and this base sequence carries out a code is

shown in the array number 1. Polymorphism according to the allele variation between individuals in the base sequence shown by the array number 3 (polymorphism) And although the variation of the amino acid sequence as the result can be considered, the base sequence and amino acid sequence of Cry j II which has such a variation are also included by this invention. 207-236 DNA of grade the amino acid sequence in which an array carries out a code -- Ala, Ile, Asn, Ile, Phe, Asn, Val, Glu, Lys, and Tyr it is -- N of matured type Cry j II It is in agreement with an end amino acid sequence (Sakaguchi, M., et al., Allergy 45, 309-312, 1990). N Since 54 amino acid of an end is rich in the hydrophobic amino acid looked at by other transit peptides and is not contained in matured type Cryj II, it is considered to be a transit peptide.

[0023] 207 DNA from grade to the 1587-1589th place of a stop codon Cry j II in which an array carries out a code -- N Ala of an end from -- C Pro of an end up to -- 460 It consists of an amino acid residue of an individual, and is considered matured type Cryj II. The amino acid sequence by which a code is carried out to the array number 4 at this base sequence in the base sequence corresponding to this matured type Cry j II is shown in the array number 2. The molecular weight on the theory of Cry j II which consists of an amino acid sequence shown in the array number 2 is 50,444Da(s). On the other hand, natural matured type Cry j II is 45KDa(s) at the SDS-polyacrylamide electrophoresis under reduction conditions (SDS-polyacrylamide gel electrophoresis). The band appears in a position (Sakaguchi, M., et al., Allergy 45, 309-312, 1990). C of this to Cry j II It is thought that the end has received processing. Moreover, Asn-X-Ser/Thr which has the possibility of N-glycosidic linkage in the amino acid sequence of Maturation Cry j II It exists.

[0024] DNA which carries out the code of Cry j II DNA containing an overall length or its part DNA which carries out the code of the protein which includes biochemical inspection, related protein, or an analogous array by carrying out an indicator by the fluorescence indicator, the radioactive indicator, or enzyme labeling It can be used as the probe for screening etc., and a primer. Moreover, it can connect with an expression vector and the protein or the peptide containing at least one epitope can also be made to discover.

[0025] ** Manifestation rCry j II of recombination Cry j II (rCry j II) Or the recombination protein or the peptide containing the epitope of at least one Cry j II includes cDNA which carries out the code of each in an expression vector, and it can be introduced into Escherichia coli, an insect cell, yeast, or mammalian, and it can obtain it by cultivating. However, the manifestation system using prokaryotic cells, such as Escherichia coli, is addition (glycosylation) of a suitable sugar chain. Since it is not carried out, it is rCry j II. It may be desirable to use eukaryotic cells, such as yeast, for a manifestation.

[0026] The example of some manifestation systems of Cry j II is shown below.

[0027] a. The promotor and RNA of manifestation T7 phage in Escherichia coli A polymerase the system (it Studier(s) F.W. --) to be used A. H. Rosenberg and J.J. Dunn and J.W. Dubendorff, "Methods in Enzymology", ed. by D. D. V. Goeddel, vol. 185, p. 60, Academic Press, New York, and 1990 Since the success percentage of a manifestation is very high, it can be used suitable for this invention. This system is the Escherichia coli host BL21 (DE3) with the polymerase gene of T7 phage. It is the system which inserted the target gene in the multi-cloning site of T7 phage promotor's lower stream of a river and which it rearranges [system], and a plasmid is introduced [system] and makes the target gene discover under IPTG existence. For example, pGEMEX-1 (Promega) etc. can be used as an expression vector.

[0028] Moreover, the system which unites the target protein with the protein which can be discovered extensive, and makes it discover is marketed, and these systems can use an affinity column for refining, and its refining efficiency is good and can use them suitable for this invention. For example, it is beta to a fusion protein. - If expression vector pUEX (Amersham) which has galactosidase is used, it is rCry j II. b. It is obtained as a fusion protein with galactosidase, and can refine efficiently by the affinity column. Moreover, the cleavage site of a blood coagulation factor Xa is introduced into the fusion zone grade, and pGEX (Pharmacia)

which has a glutathione S-transferase, pMAL (New England Biolabs, Beverly, MA) using the maltose binding protein, etc. can separate Cry j II.

[0029] b. The glycosylation of a manifestation product is possible for the system which makes manifestation yeast in yeast a host, and this is convenient to the manifestation of Cry j II which is glycoprotein. For example, as a manifestation system of the foreign protein by yeast, the method of using the Pichia yeast as a host is learned, and it can be used suitable for this invention (JP,61-108383,A, JP,61-173781,A, JP,63-44899,A, JP,1-128790,A, etc.). About the manifestation system by other yeast, they are D.Emr Scott, "Methods in Enzymology", ed.by D.V.Goeddel, vol.185, p.231, Academic Press, and New York (1990). It is explained in full detail and can be used by this invention.

[0030] c. The glycosylation of a manifestation product is possible for the system which makes a host the inside of the manifestation insect cell in an insect cell. The foreign gene manifestation system using the baculovirus is marketed (PharMingen, San Diego, CA, USA), and can be used suitable for this invention. this system -- Luckow and V.A. ** -- It is indicated by Trends in the Development of Baculovirus Expression Vector and Bio/Technology (1987 year 9 moon 11 day).

[0031] d. It can include in an expression vector with the manifestation mammals promotor (for example, metallothionein) in a mammalian cell, a virus promotor (for example, initial promotor of simian virus 40), etc., and can be made high-discovered by introducing into a mammalian cell.

[0032] (2) T of the synthetic pollinosis patient of an overlap peptide T of Cry j II which a cell recognizes Cry j II cDNA given in the array number 2 in order to solve a cell epitope on molecule level the amino acid sequence which carries out a code -- being based -- N Ala of an end from -- C Pro of an end it results -- all -- 460 The overlap peptide which covers an amino acid residue is produced. These overlap peptides are easily compoundable with the peptide automatic synthesizer unit marketed. The peptide containing at least one epitope is identified out of these overlap peptides. Especially, it is T. T which recognizes Cry j II specifically and carries out a proliferation response from a pollinosis patient's peripheral blood lymphocyte in order to identify a cell epitope It is necessary to establish a cell line. T which generally reacts for every patient Since cell epitopes differ, it is T for every patient. It is desirable to establish a cell line.

[0033] (3) the establishment Cry j II antigen of a T cell line -- specific T for establishing a cell line -- usually -- a patient's peripheral blood lymphocyte -- the bottom of existence of a Cry j II antigen, and 7 between [days] grade cultivation -- carrying out -- an antigen stimulus -- T a cell -- being activated -- further -- activation T a cell -- an antigen and an antigen presenting cell -- 7 repeat cultivating during a day several times and it carries out an antigen stimulus -- antigen specific T A cell line is producible. However, T When the cell is increasing well under existence of IL-2 of a growth factor, as for an antigen stimulus, carrying out only first is desirable. T If the abundance antigen stimulus of the cell line is carried out, it is high T of a growth ratio. A cell can be taken alternatively and it is T. The case where sufficient proliferation response is not shown depending on an epitope when identifying the peptide containing a cell epitope arises.

[0034] As an antigen to be used, although a nature type Cry j II antigen is theoretically desirable, since only a ultralow volume can be extracted from Japan cedar pollen, the mixture of recombination Cry j II (rCry j II) or an overlap peptide can also be used suitably. rCry j II What was made to discover by Escherichia coli and was refined can be used.

[0035] (4) As an establishment antigen presenting c ll of an antigen presenting cell (B cell strain), it is T. About th p ripheral blood lymphocyte of the same people as a cell line, it is mitomycin C. Processing or the thing which radiation irradiation was carri d out [thing] and made the proliferation pot ntial force lose is desirable. How v r, since the number of times of blood collecting increases, it is s If B about Epstein-Barr virus (EBV). Since it continues increasing by in vitro and becomes a lymphoblast Mr. cell strain (B c ll strain), the thing which

made it infected with a lymphocyte and made transformation start is this B. You may use a cell strain as an antigen presenting cell. B The establishment method of a cell strain is already established [the volume the technical second edition of tissue culture, 187-191 page, and on Japanese histology meeting (1988. 8.10)].

[0036] (5) T peculiar to the patient of each identification of the overlap peptide containing a T cell epitope T which a cell line recognizes The peptide containing a cell epitope is identified as follows. The meaning of "recognizing" here is T. A cellular receptor combines with an antigen epitope (including a major-histocompatibility-complex molecule) specifically, consequently it is T. Meaning that a cell is activated, the state of activation is [production of lymphokine, and] DNA. Composition [3H] It is observed by measuring the amount of incorporation of thymidine as an index. Namely, T A cell line and mitomycin C B of the same people who processed Seeding of the cell strain is carried out to 96 hole flat bottom plate, and it carries out mixed culture with an overlap peptide. [3H] The amount (cpm) of incorporation of thymidine is measured with a liquid scintillation counter. In that case [3H] The incorporation of thymidine T to each peptide since it differs by each cultivation system Cell line [3H] The amount (cpm) of thymidine incorporation Control which has not added the antigen [3H] The number (stimulation index: SI) which *(ed) in the amount (cpm) of thymidine incorporation is 2. It is T about the above. The peptide containing a cell epitope is identified. Identified T The peptides containing a cell epitope are enumerated by drawing 4.

[0037] Thus, at least one T of Cry j II of the obtained this invention The following things can b considered about the peptide containing a cell epitope. HLA Since it is thought from the analysis result (Chicz, R.M.etal.:J.Exp.Med., 178:27-47, and 1993) of a peptide that it consists of about ten to 34 amino acid residue, the length of the peptide by which combines with a class II molecule and antigen presentation is carried out is T of this invention. As for the peptide containing a cell epitope, the peptide of such length is also contained. Moreover, T for every patient [as opposed to / embellish an amino acid substitution, a deletion, or addition to the peptide of this invention, and / these ornamentation peptides] By measuring the proliferation response of a cell line, it is at least one T of Cry j II of this invention. Since producing easily the peptide containing a cell epitope and the ornamentation peptide which has this function immunologically is that this contractor can carry out easily, these ornamentation peptides are also included by this invention.

[0038] Now, the hyposensitization agent currently used by hyposensitization is the rough antigen extracted from Japan cedar pollen, and contains a lot of polysaccharide. Once there is a lot difference considerably and it starts hyposensitization, when a lot is changed, anaphylaxis is rarely started. Moreover, it is about 30% that it has seldom been improved but the curative effect of hyposensitization has also been diagnosed as higher efficacy by hyposensitization since hyposensitization treatment was started. It is a patient.

[0039] T of this invention T more than the half of the pollinosis patient among the peptides containing the epitope of a cell As for a cell line and each peptide which reacts, hyposensitization may be able to perform each of these peptides independent or above the half of the patient who treated when hyposensitization was performed using the peptide which mix d some. Moreover, since the peptide to be used is a peptide compounded chemically, it is thought that possibility of producing a side effect like anaphylaxis becomes low. For example, drawing 5 is T established from 18 pollinosis patients. The overlap peptide which a cell line recognizes, respectively "frequency of occurrence (%)" with significance index ["an average stimulus coefficient" ("— overlap — a peptide — a stimulus — d pending — T — a cell — a line — — [— three — H —] — thymidine — incorporation — an amount (cpm) — " — " — an antigen — not adding — a case — — [— three — H —] — thymidine — incorporation — an amount (cpm) — " — having divid d — a value — the average) Although value] which multiplied by " ("T c ll line which has recognized th subj ct peptide as a T c ll epitope" to "all examined T cell lines" is (%) comparatively) shows th peptid of the numbers 14, 17, 29, 38, 48, 68, 70, and

71 in drawing -- an average stimulus coefficient -- about 3.9 a significance index when it is above -- 200 It has exceeded and it is thought that it is especially effective in hyposensitization treatment.

[0040] In addition, at least one T with which this invention person clarified and was indicated to be to drawing 5 B of the after-mentioned [inside / of the peptide containing a cell epitope] Two kinds of peptides which that a cell epitope is included made clear, and the peptide which has an intersection are not contained. Therefore, the peptide of this invention is B. Since it is thought that a cell epitope is not stimulated, it thinks [that it is utilizable as a hyposensitization agent, and].

[0041] Moreover, T of this invention Internal use of the peptide containing the epitope of a cell is carried out, and it is thought possible to perform peroral-immunity tolerance. Although peroral-immunity tolerance (oral hyposensitization) is a cure under development now, the result which takes effect is beginning to be reported. For example, T of Myelin Basic Protein If internal use of the cell epitope (the peptide array 21-40 and 71-90) is carried out to a mouse, having suppressed "the Experimental Autoimmune Encephalomyelitis onset (omitting EAE)" is reported [the molecular biology of Shuichi Uenokawa, the Hisatsune ****, Toshiyuki Yamura, and peroral-immunity tolerance, a protein nucleic-acid enzyme, 39, and 2090-2101 1994 (written page 2098 right, nine to 24 lines)]. T identified also in Japan cedar pollinosis from these examples If it devises enclosing with a certain capsule etc. and internal use is carried out so that internal use of the cell epitope peptide may be carried out as it is or it may not be digested with the stomach, it may be in an immunological-tolerance state. It is specifically 12-1 before a Japan cedar pollen scattering stage. An epitope peptide is prescribed for the patient in taking orally, and the immunological-tolerance state is guided at the moon term. If it is in this state, even if Japan cedar pollen will disperse and pollen will adhere to the tunica mucosa nasi, a symptom does not come out, or a symptom is light and a bird clapper is expected.

[0042] It is T of this invention further again. The analog peptide which added ornamentation of an amino acid substitution, a deletion, or addition to the peptide containing the epitope of a cell is compounded, and it is HLA. It is T although it combines with a class II molecule. The analog peptide from which information is not transmitted is identified into a cell. These peptides will be natural T if it is used for a patient as collunarium. Since a cell epitope is checked in competition, onset prevention is expected.

[0043] In addition, a B cell epitope is also contained in the epitope as used in the field of this invention. Identification of a B cell epitope is an overlap peptide and the patient blood serum immunoglobulin E. It can carry out by well-known methods, such as reactant measurement with an antibody, and detection of prevention of combination with the patient blood serum and antigen by the overlap peptide, (refer to JP,6-69336,A). Univalent B already It is known that a c II epitope is useful to suppression of an allergic reaction. This is univalent B. A cell epitope is immunoglobulin E to which it corresponds on a mast cell or a basophilic leucocyte. immunoglobulin E combine with a molecule and according to a multiple-valued epitope It is thought that it is because formation of molecule bridge formation is checked. This invention persons compound the overlap peptide which covers all the amino acid sequences of Cry j II of this invention. These peptides and Japan cedar pollinosis patient blood serum immunoglobulin E The result which measured the reaction with an antibody by enzyme antibody technique, "A peptide Gln Cys Lys Trp Val Asn Gly Arg Glu Ile Cys (amino acid sequence 113-123)" and "Cys Thr Ser Ala Ser Ala Cys Gln Asn" (amino acid sequence 293-301) -- B A cell epitope It was shown clearly that it contains. B of such Cry j II The peptide containing a cell epitope is useful for a diagnosis, prevention, and treatment of Japan cedar pollinosis.

[0044]

[Example] Although this invention is explained in detail based on an example below, this invention is not limited to this.

[0045] <Extraction of Japan cedar pollen> Japan cedar pollen is 2 at Shizuoka Prefecture and a

Kanagawa within the prefecture. It extracted from the male which carried out flower setting to the branch of the Japan cedar cut down in the moon. Cryj II It saves by -70 **, and the Japan cedar pollen for antigenicity refining is RNA. After quick-freezing the Japan cedar pollen for manufacture in liquid nitrogen, it was saved by -70 **.

[0046] <RNA Extraction >Breiteneder ** (Int.Arch.Allergy Appl.Immunol.87:19-24 1988) adding improvement based on a method — Japan cedar pollen to RNA It extracted.

[0047] It suspends in the 15ml extraction buffer solution (100mM LiCl, 10mMNa2EDTA, 1%SDS, a 20% 2-mercaptoethanol, 100 mM Tris-HCl, pH 9.0) which ice-cooled 1g of Japan cedar pollen which carried out cryopreservation, and is 15ml phenol:chloroform:isoamyl alcohol (24:24:1) further. It added. Ize [20-30 stroke HOMOJIE] was carried out having moved this suspension to the Teflon homogenizer and turning TEX ROMPE stere by the highest rotation by the motor. Then, centrifugal operation (10,000g, 15 minutes) It separated into the water layer and the organic layer, and the water layer was obtained. a water layer — phenol [of the amount of said]: — chloroform: — isoamyl alcohol — adding — 5 a part — after between shaking and centrifugal separation (10,000g, 15 minutes) The water layer was obtained. It is the same operation 2 A time repeat, 15 moreml chloroform: Use isoamyl alcohol (24:1) and it is 1. *****. They are 4M LiCl of the amount of said to the obtained water layer. It added and was left in -20 ** overnight. The frozen solution is dissolved at a room temperature and it is centrifugal operation (20,000g, 30 minutes). Sedimentation was obtained. This sedimentation is dissolved in a little sterile distilled water, and it is 0.3. 3M CH 3 COONa of **, and pH 5.2 and 2.5 The ethanol of ** was added and it was left for 60 minutes in -20 **. Centrifugal operation (10,000g, 30 minutes) The collected dregs are remelted to a sterile distilled water, and it is all RNA. It considered as fractionation.

[0048] <composition of Japan cedar pollen mRNA manufacture-and cDNA> Japan cedar pollen — all — RNA1mg — start material — carrying out — the joint buffer solution (3 M NaCl) of the amount of said 1mM EDTA, 10 mM Tris-HCl, and pH 7.4 After adding, the span column (CLONETECH Laboratories Inc. company make —) which packed oligo dT cellulose in advance It is made to stick to CA and USA and is the elution buffer solution (1mM EDTA, 10 mM Tris-HCl, and pH 7.4). It is about 10microg by being eluted. mRNA was refined (the CLONETECH Lab.Inc. company appending protocol was followed). Then, refining mRNA 5microg Shell cDNA composition system plus (Amersham International plc. company make, Buckinghamshare, England) is used, the protocol appended is followed, and it is cDNA 4 [about]. mug It compounded.

[0049] N of the <composition of an oligonucleotide probe> Cry j II The amino acid sequence of ten residues is shown in drawing 1 A from an end. The array of cDNA expected from this amino acid sequence is drawing 1 B. Since two kinds of bases were used for the array by four places complementary as an oligonucleotide probe (Oligo CJII), it compounded as a total of 16 kinds of mixture (drawing 1 C). It is G:T in order to reduce a kind as mixture. The base pair is permitted.

[0050] <Cry j II cDNA Production of a cloning >cDNA library used cDNA cloning system lambdagt10 (Amersham International plc. company make, Buckinghamshare, and England), and performed it according to the protocol appended. Above-mentioned cDNA 1microg It included in lambdagt10 and the cDNA library was produced. Inside 5,000 [about] of about 500,000 library It is the diameter of 150mm about a clone. It wound around one plate. The probe for screening carried out the indicator of the above-mentioned oligonucleotide (Oligo CJII) by T4 polynucleotide kinase by [gamma-32P] ATP (7,000 Ci/mmol ICN Biochemicals, Inc. company make), and used it. It is the nitrocellulose filter which fixed phage DNA 5 xsubacute scleroting panencephaliti (1 xsubacute-scleroting-panencephaliti:0.18M NaCl, 10mM sodium phosphate, 1mM EDTA), 5 xFBP (1 xFBP:0.02% Ficoll, 0.02% cow serum albumin, 0.02% polyvinyl pyrrolidone), 0.3%SDS, and 100 mug/ml tRNA It is 1 48 degrees C to the included solution. It pre hybridized by dipping beyond time. It dips in this solution which newly prepared the nitrocellulose filter after this, and is 32P. The probe (Oligo CJII) which carried out the label was added, and

hybridization was performed at 48 degrees C overnight. They are 6 xSSC (1 xSSC:0.15M NaCl, 0.015M sodium citrate) and 0.1%SDS about a filter after this. It is 5 48 degrees C the room temperature of 30 degrees C with the included solution. Autoradiography was performed after part-washing. 4 The strong signal of an individual is detected and it is one of phage DNA of it. It extracts and is a restriction enzyme EcoRI. When cut, it is DNA of about 1.7 Kbp(s). It became clear that the fragment is inserted. Sub cloning of the insert was carried out to pUC118, the delay SHON mutant was produced using the kilo sequence DERESHON kit (TAKARA SHUZO CO., LTD. make), and it used for the determination of all base sequences. The base sequence performed the primer extension reaction using the synthetic primer and the coloring matter indicator dideoxy terminator, and determined it by reading by the automatic sequencer (model 370A, Applied Biosystems, and Japan). All the determined cDNA base sequences are shown in the array number 5. moreover, the base sequence which carries out the code of the maturation Cry j II for the base sequence of only an open reading frame to the array number 3 (the amino acid sequence in which this base sequence carries out a code — the array number 1) is shown in the array number 4 (the amino acid sequence in which this base sequence carries out a code — the array number 2)

[0051] <Manifestation by the Escherichia coli of recombination Cry j II> Promega It is 1TEscherichia coli expression vector pGEMEX-7 promotor and T7 gene10 which are marketed from the shrine. It has coding sequence and T7 terminator, and is T7 gene10 about an open reading frame. It inserts in a down-stream multi-cloning site, and is T7. It is the vector which performs a high manifestation by introducing into the Escherichia coli (Example BL21 (DE3)) which discovers RNA polymerase. Cry j II cDNA It digests by BamHI (the adapter connected with the ends of cDNA includes a BamHI site), cDNA fragmentation is started, and it is BamHI of pGEMEX-1. It includes in a site and is expression vector pEXCJII of Cry j II. It built. pEXCII is T7 gene10. The fusion protein (T7 Cry j II, 73kD) of a manifestation product (23kD) and Cry j II protein (50kD) may be discovered. It is Escherichia coli BL21 (DE3) about pEXCII. The introduced transformant is cultivated and it is T7 at IPTG. RNA polymerase was guided and Cry j II was discovered. It is SDS about the cell extract of the discovered Escherichia coli. It applied to polyacrylamide gel electrophoresis. BL21 (DE3) holding pEXCII **** — T7 Cry j II of about 73 kD(s) ** — the band considered was seen however, BL21 (DE3) holding pGEMEX-1 of contrast Or old stock BL21 (DE3) **** — these bands were not seen

[0052] <Cry j II and T7 gene10 Reactant >T7 Cry j II with the Japan cedar pollinosis patient blood serum of fusion protein (T7Cry j II) About the extract of the discovered Escherichia coli, it is SDS. Millipore after carrying out polyacrylamide gel electrophoresis Western blotting (West rn Blotting) is carried out to a shrine PVDF film, and it is the Japan cedar pollinosis patient 5. People and healthy people 3 Reactivity with people's blood serum was examined. BL21 and T7 gene10 which hold pGEMEX-1 as contrast Cry j I Nature type Cry j I refined from the extract of BL21 which discovered the fusion protein (T7Cry j I), and Japan cedar pollen The blot was carried out simultaneously and the reaction was investigated. It is 2 as shown in drawing 2 . People's patient blood serum is T7 Cry jII. It reacted. 2 People's patient blood serum is T7 Cry j II and a nature type. Although it has reacted to Cry j I, it has not reacted to BL21 extract and T7 Cry j I holding pGEMEX-1. These results to T7Cry j II is immunoglobulin E in a Japan cedar pollinosis patient blood serum. Having the antigenicity which reacts was checked.

[0053] Composition of a <composition of overlap peptide> exaggerated peptide was performed using Peptide Synthesizer PSSM -8 (Shimadzu make). a basis [primary structure / of Cry j II / which is shown in the array number 2] — carrying out — N an end side — 55th Ala from — starting — C Pro of an end up to — 90 kinds of ov rlap peptides of 15 **** containing the overlap portion of ten residues w re compounded 1 charact r code of amino acid is used for drawing 3 -6, and all the compound overlap peptides are shown.

[0054] <B the peripheral blood lymphocyte (1x10⁶) obtain d by th establishm nt >Ficoll-Paque specific gravity centrifugation of a cell strain — about — Epstein-Barr virus (EBV) of 1 x10⁶

PFU (plaque forming units) -- 37 degrees C -- 1 Time incubation was carried out and the virus was infected with the cell. This virus infection cell is moved to 24 well cultivation plate, and it is 100 ng/ml cyclosporin A. It is 2 under existence. It is B if it cultivates before or after a week. A cell colony appears. It divided into the half at this time, and it planted in the n w well and inherited. If this operation is repeated successively and subculture is performed, it is B in which self-multiplication is possible. A cell may appear. This self-multiplication B 25cm2 after carrying out IKUSUPANDO (expand) of the cell of the well containing a cell and checking proliferation It moves to a culture flask, cultivation is performed for further 30 - 50 days, and it is EBV. B which it transformed (EBV-transformed) The cell strain was obtained. B Cryopreservation of a part of cell strain was carried out.

[0055] <Cry j II antigen specific T It saved in liquid nitrogen until it isolated and used it by the Ficoll-paque specific gravity centrifugation usually used in the lymphocyte from the 18 establishment > Japan cedar pollinosis patient peripheral blood of a cell line. a Japan cedar pollinosis patient's peripheral blood lymphocyte (4 x106 individual) -- 20% of self plasma of 2 ml RPMI-1640 added suspended, and it was made discovered by 10microg [/ml] Escherichia coli, and refined -- rearranging -- a Cry j II antigen -- 24 hole cultivation plate top -- 7-8 It cultivated during the day. T activated in response to the Cry j II antigen stimulus (a ***** reaction and blastogenesis) It is 5 Unit/ml when the cell has checked under the microscope. IL-2 were added and it cultivated overnight. They are 20 Unit/ml IL-2 from the next day, and 20%. RPMI-1640 which added the Homo sapiens AB type blood serum (commercial elegance) It is 9, replacing culture medium with every day. It cultivated during the day. Increased T which recognizes a Cry j II antigen specifically at this time Cryopreservation of a part of cell line was carried out. Furthermore, it is T. It is a cell line in the above-mentioned culture medium 4 It cultivated during the day and considered as the cell for identification of an epitope.

[0056] <T T established from the identification >18 person pollinosis patient of the exaggerated peptide containing a cell epitope It cultivates with a Japan cedar pollen-allergen overlap peptide about a cell line, respectively, and is Cry j II antigen specific T. The peptide containing a cell epitope was identified.

[0057] T Cultivation B established from the same patient as a cell line It is 50microg [/ml] mitomycin C about a cell strain. It processes for 30 minutes and is a cell RPMI-1640 4 Time washing was carried out. This B After carrying out seeding (5 x104 / well) of the cell to 96 hole flat bottom plate (96-well flat-bottomed plate), it added to each well and Cry j II (the 25microg [/ml] last concentration) or each overlap peptide (last concentration 0.5 muM) was cultivated for about 60 - 90 minutes. T Carry out seeding of the cell line (2x104 / well) to each well, and it is after 48-hour cultivation and 0.5. It added to the well and mul/Ci [3 H] thymidine was cultivated for further 16 hours. The uptake of the cell was carried out on the glass filter using the cell harvester, and after drying, the count (cpm) of [3 H] thymidine incorporated in the cell was measured with the liquid scintillation counter.

[0058] Measurement is performed by triplicate culture. a result T by overlap peptide stimulus The amount (cpm) of [3 H] thymidine incorporation of a cell line It computes by the stimulus coefficient (stimulation index; SI) which is the value broken by the amount (cpm) of [3 H] thymidine incorporation when not adding an antigen (control). SI is 2. About the overlap peptide which showed the above value, it is T. The exaggerated peptide containing a cell epitope was identified. Drawing 7 and drawing 8 are Cry j II antigen specific T established from 18 pollinosis patients, respectively. At least one T which at least one kind of a cell line recognizes The peptide containing a cell epitope is shown. Moreover, drawing 9 shows th "average stimulus coefficient" (average of the stimulus coefficient obtained by two or more experim nts) and "the frequency of occurrence (%)" of all overlap peptides, and the "significance index."

[0059]

[Effect of the Inv ntion] The peptide and T containing at least one epitope of Cry j II of this invention The peptide containing a cell epitope is us ful for a diagnosis, prevention, and

treatment of Japan cedar pollinosis.

[0060] It is HLA further again. Class It is T although it combines with a mol cul . It is also possible to compound an analog peptide from which information is not transmitted to a cell, and to use these peptides for the Japan cedar pollinosis onset prevention by competitive inhibition.

[0061]

[Layout Table]

array number: -- length [of one array]: -- mold [of 514 arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- protein array: -- Met Ala Met Lys Leu Ile Ala Pro Met Ala Phe Leu Ala Met Gln Leu 5 10 15 Ile Ile Met Ala Ala Ala Glu Asp Gln Ser Ala Gln Ile Met Leu Asp 20 25 30 Ser Val Val Glu Lys Tyr Leu Arg Ser Asn Arg Ser Leu Arg Lys Val 35 40 45 Glu His Ser Arg His Asp Ala Ile Asn Ile Phe Asn Val Glu Lys Tyr 50 55 60 Gly Ala Val Gly Asp Gly Lys His Asp Cys Thr Glu Ala Phe Ser Thr 65 70 75 80 Ala Trp Gln Ala Ala Cys Lys Asn ProSer Ala Met Leu Leu Val Pro 85 90 95 Gly Ser Lys Lys Phe Val Val Asn Asn Leu Phe Phe Asn Gly Pro Cys 100 105 110 Gln Pro His PheThr Phe Lys Val Asp Gly Ile Ile Ala Ala Tyr Gln 115 120 125 Asn Pro Ala Ser Trp Lys Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys 130 135 140 Leu Thr Gly Phe Thr Leu Met Gly Lys GlyVal Ile Asp Gly Gln Gly 145 150 155 160 Lys Gln Trp Trp Ala Gly GlnCys Lys Trp Val Asn Gly Arg Glu Ile 165 170 175 Cys Asn Asp Arg AspArg Pro Thr Ala Ile Lys Phe Asp Phe Ser Thr 180 185190 Gly Leu Ile Ile Gln Gly Leu Lys Leu Met Asn Ser Pro Glu Phe His 195 200 205 Leu Val Phe Gly Asn Cys Glu Gly Val Lys Ile Ile Gly Ile Ser Ile 210 215 220 Thr Ala Pro Arg Asp Ser Pro Asn Thr Asp Gly Ile Asp Ile Phe Ala 225 230 235 240 Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile Gly Thr Gly Asp Asp 245 250 255 Cys Val Ala Ile Gly Thr GlySer Ser Asn Ile Val Ile Glu Asp Leu 260 265270 Ile Cys Gly Pro Gly His Gly IleSer Ile Gly Ser Leu Gly Arg Glu 275 280 285 Asn Ser Arg Ala Glu Val Ser Tyr Val His Val Asn Gly Ala Lys Phe 290 295 300 Ile Asp Thr Gln Asn Gly Leu Arg Ile Lys Thr Trp Gln Gly Gly Ser 305 310 315 320 Gly Met Ala Ser His Ile Ile Tyr Glu Asn Val Glu Met Ile Asn Ser 325 330 335 Glu Asn Pro Ile Leu Ile Asn Gln Phe-Tyr-Cys-Thr-Ser-Ala-Ser-Ala 340 345 350 Cys Gln Asn Gln Arg Ser Ala Val Gln-Ile-Gln-Asp-Val-Thr-Tyr-Lys 355 360365 Asn Ile Arg Gly Thr Ser Ala Thr Ala Ala Ile Gln Leu Lys Cys 370 375 380 Ser Asp Ser Met Pro Cys Lys Asp Ile Lys Leu Ser Asp Ile Ser Leu 385 390 395 400 Lys Leu Thr Ser Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn Ala Asn 405 410 415 Gly Tyr Phe Ser Gly His Val Ile Pro Ala Cys Lys Asn Leu Ser Pro 420 425 430 Ser Ala Lys Arg Lys Glu Ser Lys Ser His Lys His Pro Lys Thr Val 435 440 445 Met Val Glu Asn Met Arg Ala Tyr Asp Lys Gly Asn Arg Thr Arg Ile 450 455 460 Leu Leu Gly Ser Arg Pro Pro Asn Cys Thr Asn Lys Cys His Gly Cys 465 470 475 480 Ser Pro Cys Lys Ala Lys Leu Val Ile Val His Arg Ile Met Pro Gln 485 490 495 Glu Tyr Tyr Pro Gln Arg Trp Ile Cys Ser Cys His Gly Lys Ile Tyr 500 505 510His Pro array number: -- length [of two arrays]: -- mold [of 460 arrays]: -- amino acid topology: -- kind [of straight chain-like array]: -- protein array: -- Ala Ile Asn Ile Phe Asn Val Glu Lys Tyr Gly Ala Val Gly Asp Gly 5 10 15 Lys His Asp Cys Thr Glu Ala Phe Ser Thr Ala Trp Gln Ala Ala Cys 20 25 30 Lys Asn Pro Ser Ala Met Leu Leu Val Pro Gly Ser Lys Lys Phe Val 35 40 45 Val Asn Asn Leu Phe Phe AsnGly Pro Cys Gln Pro His Phe Thr Phe 50 55 60 Lys Val Asp Gly Ilelle Ala Ala Tyr Gln Asn Pro Ala Ser Trp Lys 65 70 75 80 Asn Asn Arg Ile Trp Leu Gln Phe Ala Lys Leu Thr Gly Phe Thr Leu 85 90 95 Met Gly Lys Gly Val Ile Asp Gly Gln Gly Lys Gln Trp Trp Ala Gly 100 105 110 Gln Cys Lys Trp Val Asn Gly Arg Glulle CysAsn Asp Arg Asp Arg 115 120 125 Pro Thr Ala IleLys PheAsp Phe Ser Thr Gly Leu I le-Ile-Gln-Gly 130 135 140 Leu Lys Leu Met Asn Ser Pro Glu Phe-His-Leu-Val-Phe-Gly Asn Cys 145 150 155 160 Glu Gly Val Lys Ile Ile Gly Ile Ser Ile Thr Ala Pro Arg-Asp Ser 165 170175 Pro Asn Thr Asp Gly Ile Asp Ile Phe Ala Ser Lys Asn Phe His L u 180 185 190 Gln Lys Asn Thr Ile Gly Thr Gly Asp Asp Cys Val Ala Ile Gly Thr 195 200 205 Gly S r Ser Asn Il Val Ile Glu Asp Leu Ile Cys Gly Pro Gly His 210 215 220 Gly Ile Ser Ile Gly S r Leu Gly Arg Glu Asn Ser Arg Ala Glu Val 225 230 235 240 Ser Tyr Val His Val Asn Gly Ala Lys Phe Il Asp Thr Gln Asn Gly 245 250 255 Leu Arg Ile Lys Thr Trp Gln Gly Gly Ser Gly Met Ala Ser His Ile 260 265 270 Ile Tyr Glu Asn Val Glu Met Il Asn Ser Glu Asn Pro Ile Leu Ile 275 280 285 Asn Gln Phe Tyr Cys Thr Ser Ala Ser Ala Cys Gln Asn

Gln Arg Ser 290 295 300 Ala Val Gln Ile Gln Asp Val Thr Tyr Lys Asn Ile Arg Gly Thr Ser 305
 310 315 320 Ala Thr Ala Ala Ala Ile Gln Leu Lys Cys Ser Asp Ser Met Pro Cys 325 330 335 Lys
 Asp Ile Lys Leu Ser Asp Ile Ser Leu Lys Leu Thr Ser Gly Lys 340 345 350 Ile Ala Ser Cys Leu
 Asn Asp Asn Ala Asn Gly Tyr Phe Ser Gly His 355 360 365 Val Ile Pro Ala Cys Lys Asn Leu Ser
 Pro Ser Ala Lys Arg Lys Glu 370 375 380 Ser Lys Ser His Lys His Pro Lys Thr Val Met Val Glu
 Asn Met Arg 385 390 395 400 Ala Tyr Asp Lys Gly Asn Arg Thr Arg Ile Leu Leu Gly Ser Arg Pro
 405 410 415 Pro Asn Cys Thr Asn Lys Cys His Gly Cys Ser Pro Cys Lys Ala Lys 420 425 430
 Leu Val Ile Val His Arg Ile Met Pro Gln Glu Tyr Tyr Pro Gln Arg 435 440 445 Trp Ile Cys Ser
 Cys His Gly Lys Ile Tyr His Pro 450 455 460 array number: — length [of three arrays]: — mold
 [of 1542 arrays]: — nucleic-acid-topology: — kind [of straight chain-like array]: — cDNA to
 mRNA Array : ATGGCCATGA AATTAATTGC TCCAATGGCC TTTCTGGCCA TGCAATTGAT
 TATAATGGCG 60 GCAGCAGAAG ATCAATCTGC CCAAATTATG TTGGACAGTG
 TTGTCGAAAA ATATCTTAGA 120 TCGAATCGGA GTTTAAGAAA AGTTGAGCAT
 TCTCGTCATG ATGCTATCAA CATCTTCAAT 180 GTGGAAAAAT ATGGCGCAGT
 AGGCGATGGA AAGCATGATT GCACTGAGGC ATTTTCAACA 240 GCATGGCAAG
 CTGCATGCAA AAACCCATCA GCAATGTTGC TTGTGCCAGG CAGCAAGAAA 300
 TTTGTTGTAA ACAATTTGTT CTTCAATGGG CCATGTCAAC CTCACCTTAC TTTTAAGGTA
 360 GATGGGATAA TAGCTGCGTA CCAAAATCCA GCGAGCTGGA AGAATAATAG
 AATATGGTTG 420 CAGTTTGCTA AACTTACAGG TTTTACTCTA ATGGGTAAAG
 GTGTAATTGA TGGGCAAGGA 480 AAACAATGGT GGGCTGGCCA ATGTAAATGG
 GTCAATGGAC GAGAAATTTG CAACGATCGT 540 GATAGACCAA CAGCCATTAA
 ATTCGATTTT TCCACGGGTC TGATAATCCA AGGACTGAAA 600 CTAATGAACA
 GTCCCGAATT TCATTTAGTT TTTGGGAATT GTGAGGGAGT AAAAATCATC 660
 GGCATTAGTA TTACGGCACC GAGAGACAGT CCTAACACTG ATGGAATTGA TATCTTTGCA
 720 TCTAAAACT TCACTTACA AAAGAACACG ATAGGAACAG GGGATGACTG
 CGTCGCTATA 780 GGCACAGGGT CTTCTAATAT TGTGATTGAG GATCTGATTT
 GCGGTCCAGG CCATGGAATA 840 AGTATAGGAA GTCTTGGGAG GGAAAACTCT
 AGAGCAGAGG TTTCATACGT GCACGTAAAT 900 GGGGCTAAAT TCATAGACAC
 ACAAATGGA TTAAGAATCA AAACATGGCA GGGTGGTTCA 960 GGCATGGCAA
 GCCATATAAT TTATGAGAAT GTTGAAATGA TAAATTCGGA GAACCCCATATA 1020
 TTAATAAATC AATTCTACTG CACTTCGGCT TCTGCTTGCC AAAACCAGAG GTCTGCGGTT
 1080 CAAATCCAAG ATGTGACATA CAAGAACATA CGTGGGACAT CAGCAACAGC
 AGCAGCAATT 1140 CAACTTAAGT GTAGTGACAG TATGCCCTGC AAAGATATAA
 AGCTAAGTGA TATATCTTTG 1200 AAGCTTACCT CAGGGAAAAT TGCTTCCTGC
 CTTAATGATA ATGCAAATGG ATATTTTCAAGT 1260 GGACACGTCA TCCCTGCATG
 CAAGAATTTA AGTCCAAGTG CTAAGCGAAA AGAATCTAAA 1320 TCCCATAAAC
 ACCCAAAAAC TGTAATGGTT GAAAATATGC GAGCATATGA CAAGGGTAAC 1380
 AGAACACGCA TATTGTTGGG GTCGAGGCCT CCGAATTGTA CAAACAAATG TCATGGTTGC
 1440 AGTCCATGTA AGGCCAAGTT AGTTATTGTT CATCGTATTA TGCCGCAGGA
 GTATTATCCT 1500 CAGAGGTGGA TATGCAGCTG TCATGGCAAA ATCTACCATC CA 1542
 array number: — length [of four arrays]: — mold [of 1380 arrays]: — nucleic-acid-topology:
 — kind [of straight chain-like array]: — cDNA to mRNA array: — GCTATCAACA
 TCTTCAATGT GGAAAAATAT GGCGCAGTAG GCGATGGAAA GCATGATTGC 60
 ACTGAGGCAT TTTCAACAGC ATGGCAAGCT GCATGCAAAA ACCCATCAGC AATGTTGCTT
 120 GTGCCAGGCA GCAAGAAATT TGTTGTAAAC AATTTGTTCT TCAATGGGCG
 ATGTCAACCT 180 CACTTTACTT TTAAGGTAGA TGGGATAATA GCTGCGTACC
 AAAATCCAGC GAGCTGGAAG 240 AATAATAGAA TATGGTTGCA GTTTGCTAAA
 CTTACAGGTT TTAATCTAAT GGGTAAAGGT 300 GTAATTGATG GGCAAGGAAA
 ACAATGGTGG GCTGGCCAAT GTAAATGGGT CAATGGACGA 360 GAAATTTGCA
 ACGATCGTGA TAGACCAACA GCCATTAAAT TCGATTTTTC CACGGGTCTG 420ATAATCC
 AAG-GACTGAACT-AATGAACAGT CCCGAATTTT-ATTTAGTTTT TGGAATTGT

480GAGGGAGTAA AAATCATCGG-CATTAGTATT ACGGCACCGA-GAGACAGTCC
 TAACACTGAT 540GGAATTGATA TCTTTGCATC-TAAAACTTT
 CACTTACAAA-AGAACACGAT AGGAACAGGG 600GATGACTGCG
 TCGCTATAGG-CACAGGGTCT TCTAATATTG-TGATTGAGGA TCTGATTTGC
 660GGTCCAGGCC ATGGAATAAG TATAGGAAGT CTTGGGAGGG AAAACTCTAG
 AGCAGAGGTT 720TCATACGTGC ACGTAAATGG GGCTAAATTC ATAGACACAC
 AAAATGGATT AAGAATCAAA 780ACATGGCAGG GTGGTTCAGG CATGGCAAGC
 CATATAATTT ATGAGAATGT TGAAATGATA 840AATTCGGAGA ACCCCATATT
 AATAAATCAA TTCTACTGCA CTTCGGCTTC TGCTTGCCAA 900AACCAGAGGT
 CTGCGGTTCA AATCCAAGAT GTGACATACA AGAACATACG TGGGACATCA
 960GCAACAGCAG CAGCAATTCA ACTTAAGTGT AGTGACAGTA
 TGCCCTGCAAAGATATAAAG 1020 CTAAGTGATA TATCTTTGAA GCTTACCTCA
 GGGAAAATTG CTTCTGCT TAATGATAAT 1080 GCAAATGGAT ATTCAGTG
 ACACGTCATC CCTGCATGCA AGAATTTAAG TCCAAGTGCT 1140 AAGCGAAAAG
 AATCTAAATC CCATAAACAC CCAAAAATG TAATGGTTGA AAATATGCGA 1200
 GCATATGACA AGGGTAACAG AACACGCATA TTGTTGGGGT CGAGGCCTCC GAATTGTACA
 1260 AACAAATGTC ATGGTTGCAG TCCATGTAAG GCCAAGTTAG TTATTGTTCA
 TCGTATTATG 1320 CCGCAGGAGT ATTACCTCA GAGGTGGATA TGCAGCTGTC
 ATGGCAAAT CTACCATCCA 1380 array number: — length [of five arrays]: — mold [of
 1733 arrays]: — nucleic-acid-topology: — kind [of straight chain-like array]: — cDNA to
 mRNA array: — AGTTGAGTTC GAGACAAGTA TAGAAAGAAT TTTCTTTTAT TAAAATGGCC
 ATGAAATTAA 60 TTGCTCCAAT GGCCTTTCTG GCCATGCAAT TGATTATAAT
 GGCGGCAGCA GAAGATCAAT 120 CTGCCCAAAT TATGTTGGAC AGTGTGTGCG
 AAAAATATCT TAGATCGAAT CGGAGTTTAA 180 GAAAAGTTGA GCATTCTCGT
 CATGATGCTA TCAACATCTT CAATGTGGAA AAATATGGCG 240 CAGTAGGCGA
 TGGAAGCAT GATTGCACTG AGGCATTTTC AACAGCATGG CAAGCTGCAT 300
 GCAAAAACCC ATCAGCAATG TTGCTTGTGC CAGGCAGCAA GAAATTTGTT GTAAACAATT
 360 TGTTCTTCAA TGGGCCATGT CAACCTCACT TTAATTTTAA GGATAGATGGG
 ATAATAGCTG 420 CGTACCAAAA TCCAGCGAGC TGGAAGAATA ATAGAATATG
 GTTGCAAGTTT GCTAAACTTA 480 CAGGTTTTAC TCTAATGGGT AAAGGTGTAA
 TTGATGGGCA AGGAAAACAA TGGTGGGCTG 540 GCCAATGTAA ATGGGTCAAT
 GGACGAGAAA TTTGCAACGA TCGTGATAGA CCAACAGCCA 600 TTAAATTCGA
 TTTTCCACG GGTCTGATAA TCCAAGGACT GAACTAATG AACAGTCCCG 660
 AATTCATTT AGTTTTTGGG AATTGTGAGG GAGTAAAAAT CATCGGCATT AGTATTACGG
 720 CACCGAGAGA CAGTCCTAAC ACTGATGGAA TTGATATCTT TGCATCTAAA
 AACTTTCATC 780 TACAAAAGAA CACGATAGGA ACAGGGGATG ACTGCGTCGC
 TATAGGCACA GGGTCTTCTA 840 ATATTGTGAT TGAGGATCTG ATTTGCGGTC
 CAGGCCATGG AATAAGTATA GGAAGTCTTG 900 GGAGGGAAAA CTCTAGAGCA
 GAGGTTTCAT ACGTGCACGT AAATGGGGCT AAATTCATAG 960 ACACACAAAA
 TGGATTAAGA ATCAAAACAT GGCAGGGTGG TTCAGGCATG GCAAGCCATA 1020
 TAATTTATGA GAATGTTGA A-ATGATAAATT-CGGAGAACCC CATATTAATA-AATCAATTCT
 1080ACTGCACTTC GGCTTCTGCT TGCCAAAACC-AGAGGTCTGC
 GGTTCAAATC-CAAGATGTGA 1140CATACAAGAA CATACGTGGG
 ACATCAGCAA-CAGCAGCAGC AATTCAACTT-AAGTGTAGTG 1200ACAGTATGCC
 CTGCAAAGAT ATAAAGCTAA-GTGATATATC TTTGAAGCTT-ACCTCAGGGA 1260
 AAATTGCTTC CTGCCTTAAT GATAATGCAA ATGGATATTT CAGTGGACAC GTCATCCCTG
 1320 CATGCAAGAA TTTAAGTCCA AGTGCTAAGC GAAAAGAATC TAAATCCCAT
 AAACACCCAA 1380 AAATGTAAT GGTTGAAAAT ATGCGAGCAT ATGACAAGGG
 TAACAGAACA CGCATATTGT 1440 TGGGGTCGAG GCCTCCGAAT TGTAACAACA
 AATGTCATGG TTGCAGTCCA TGTAAGGCCA 1500 AGTTAGTTAT TGTTTCATCGT
 ATTATGCCGC AGGAGTATTA TCCTCAGAGG TGGATATGCA 1560 GCTGTCATGG

CAAAATCTAC CATCCATAAT GAGATACATT GAAACTGTAT GTGCTAGTGA 1620
ATATTCTTGT GGTACAATAT TAGAACTGAT ATTGAAAATA AATCATCAAT GTTTCTAAGG
1680 CATTTATAAT AGATTATATT AATGGTTCAA AAAAAAAAAA AAAAAAAAAA AAA 1733

[Translation done.]

*** NOTICES ***

Japan Patent Office is not responsible for any damages caused by the use of this translation.

1. This document has been translated by computer. So the translation may not reflect the original precisely.
2. **** shows the word which can not be translated.
3. In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1] N of Japan cedar pollen-allergen Cry j II Amino acid sequence of an end to ten residues (A) . N of Japan cedar pollen-allergen Cry j II DNA expected from the amino acid sequence of an end to ten residues Array (B) . DNA of the probe for screening cDNA which carries out the code of Japan cedar pollen-allergen Cry j II Array (C) .

[Drawing 2] T7 Cry j II It is antigenicity 2 The result identified by the Western blot technique is shown using the blood serum of the Japan cedar pollinosis patient of a name. For BL21 (DE3) holding pMGEMEX-1 (negative control), and a lane 2, BL21 (DE3) which discovered T7 Cry j I, and a lane 3 are [a lane 1] T7Cry j II. A lane 4 is discovered BL21 (DE3) and Cry j I refined from Japan cedar pollen. It is shown, respectively. A B It is only that the patients in whom a blood serum originates differ, and others are the same.

[Drawing 3] The overlap peptide which covers all the amino acid sequences of Cry j II is shown.

[Drawing 4] The overlap peptide which covers all the amino acid sequences of Cry j II is shown.

[Drawing 5] The overlap peptide which covers all the amino acid sequences of Cry j II is shown.

[Drawing 6] The overlap peptide which covers all the amino acid sequences of Cry j II is shown.

[Drawing 7] At least one T of Cry j II The peptide containing a cell epitope is shown.

[Drawing 8] At least one T of Cry j II The peptide containing a cell epitope is shown.

[Drawing 9] T specific to the Cry j II allergen established from 18 Japan cedar pollinosis patients The significance index of the overlap peptide which a cell line recognizes, respectively is shown.

[Translation done.]